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LUNG CLEARANCE OF MONO- AND DI-DISPERSE AEROSOLS DETERMINED BY PROFILE SCANNING AND WHOLE-BODY COUNTING

A study on normal and SO_2 exposed rabbits

By

BO HOLMA

STOCKHOLM 1967

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INTRODUCTION

The lung's ability to rid itself of deposited material—lung clearance—is one of the mechanisms which protects the organism from injurious effects of air pollutants. This defence mechanism, comprising various physiological functions, thus determines the time during which different agents can exert their effect in the lung. The transport of secretions assisted by ciliary motility is a major mechanism responsible for elimination of foreign matter from the lung. Elimination is also effected by phagocytosis and by diffusions or direct penetration of the material in question to the interstitial tissues, where it can accumulate in the lymphatics and/or enter the bloodstream. Diffusion is most important for soluble substances, and the other mechanisms are more concerned with the elimination of solid or semi-insoluble substances. Lung clearance is also affected by the physiological and pathological status of the organism.

Numerous investigations of lung clearance have been made but many problems remain unanswered. Some of the results are contradictory and the discrepancies have been explained in terms of differences in methodology, experimental animal used, observation time etc. Generally speaking, however, it can be said that lung clearance has an initial rapid phase and a slower subsequent course. However, the initial phase has not been adequately investigated due to the lack of suitable methods. There are results which indicate that large particles have a faster clearance than small ones, although in a few experiments the reverse was reported. The intra- and interindividual variation of lung clearance has not been investigated except for one study that concerned only the alveolar clearance of submicronic particles. The effect of gaseous air pollutants on lung clearance has been reported in only one study performed on sacrificed animals.

The above questions are considered in the present study with monodisperse aerosols. The dependence of lung clearance on particle size was investigated using two discrete particle sizes simultaneously on one and the same animal. The intra- and interindividual variations of the two phases of this lung function and the effect of a gaseous air pollutant (SO_2) on lung clearance in live animals were also investigated.

Chapter 1 surveys data from the literature concerning important findings about the mechanisms involved in lung clearance and also deals with various methods that have been used to study lung clearance with particular reference to methods for external measurement. This is followed by a presentation of the problem investigated and the arrangement of the experiments conducted by the present author. A subsequent chapter deals with the production and characteristics of the test aerosols used. A separate chapter is devoted to the technique of exposure and this is followed by a presentation of the measuring technique, the validity of the apparatus for these studies, and methods for evaluating the measurements obtained. In conclusion, an account is given of some

applications of the present measuring technique in lung clearance studies, with comments on the significance of particle size in test aerosols and the importance of intra- and interindividual variations in this lung function. The suitability of the method for studying effects on lung clearance is illustrated using rabbits exposed to SO_2.

GENERAL BACKGROUND

Definitions

In keeping with the definitions laid down by the Subcommittee on Inhalation Hazards (62) *deposition* is used here to refer to the amount of inhaled material that remains in the respiratory tract after expiration By *retention* is meant the amount or fraction of the deposited material that remains in the respiratory tract at any given time Thus deposition is the same as initial retention The transport of material out of the lungs via the trachea is termed *clearance* and the removal of material to other tissues *translocation*

In the present work, the term *lung clearance* refers to the clearance of the lower respiratory tract i e the tracheobronchial and pulmonary compartments as defined by the Task Group on Lung Dynamics (96)

By *aerosol* is meant solid particles o liquid drops distributed in air and of such a particle size that gravitational and diffusional forces impart sufficient stability for the system to be classified as an air suspension Aerosols are described as *mono disperse* if the particles are sufficiently similar in size for them to behave—as a unit and concerning the parameter being investigated—in a manner typical of the mean particle size A *di disperse* aerosol *is one containing a mix*ture of two different sizes of monodisperse particles

The measurement data concerning the size of aerosol particles is given in terms of their *count median diameter* (CMD) unless otherwise stated The abbreviation MMAD stands for the particles *mass median aerodynamic diameter* i e the mass of a unit density sphere with the same settling velocity as the particle in question (96)

Lung clearance

There is a lack of detailed data concerning lung clearance mechanisms (46 96) A brief description will therefore be given of generally accepted principles and experimental findings concerning the complex of various functions that are known or presumed to be involved in this function of the lung The references given here are by way of illustration and do not pretend to be complete This field has been systematically reviewed by the Task Group on Lung Dynamics (96) and by Hatch & Cross (47)

Even though the mechanisms involved in the total clearance process have not always been investigated in an entirely satisfactory manner lung clearance is considered to comprise at least the following functions transport of secretion promoted by ciliary activity endocytosis resorption and direct penetration of particles through the lung parenchyma

However clearance is not a uniform process in the different parts of the respiratory tract Alveolar clearance for instance differs entirely from the clearance mechanisms in the nasal buccopharyngeal and tracheobronchial sections

Alveolar clearance mechanisms

Although the mechanisms responsible for the transport of solid particles deposited in the alveoli are somewhat obscure, it is known that the greater part will be carried to the mucociliary transport mechanism of the bronchi, while a smaller fraction finds its way into the interstitial tissue and lymphatics from where a minor quantity enters the bloodstream. Part of the material may be translocated directly to the blood depending on its solubility in vivo.

It is not clear which mechanism or mechanisms are responsible for transporting the particles from the alveoli to the ciliated parts and no generally accepted solution has yet been found to certain questions raised by the earliest research in this field. In particular there is the problem of the mechanisms that contribute to the transport of particles to the interstitial tissue with the related questions concerning the structure of the alveolar walls, the origin of the phagocytes in the respiratory tracts and their role in this translocation.

Some investigations suggest that particles penetrate straight into the interstitial tissue without the help of phagocytes; this has been reported by Gross & Westrick (43). [illegible] phagocytosis is rather a protective mechanism since it makes it more difficult for the particles to penetrate the lung membranes. Other authors, e.g. Cassaret (18), consider that particles do penetrate to [illegible] with the help of phagocytic cells.

A [illegible] here concerns the origin of phagocytic cells, particularly the macrophages. A hematopoietic origin for the lung macrophages appears to be strongly supported by the results of [illegible] et al. (80) from experiments [illegible] in which the [illegible] identified by marker chromosomes. They demonstrated that about 2/3rds of the lung macrophages displayed the same specific chromosomes that indicated their hematopoietic origin.

Gross (42) argued that alveolar clearance is dependent upon phagocytosis of the macrophages. This was demonstrated by LaBelle & Brieger (65) in a study on the rat in which they found that the elimination of carbon particles was correlated to the number of free phagocytes in the lungs. Stecker (93) showed that cytotoxic substances such as quartz elicit a marked increase in alveolar cells and in the degree of mitosis among these, whereas such effects are not obtained with "inert" material. Quartz and other cytotoxic particles that undergo phagocytosis lead to destruction of the phagocytes and produce changes in the alveolar walls that enable these particles to penetrate to the interstitial tissue and the lymphatics (60, 93, 103). "Inert" particles however do not destroy the phagocytes (89) and can be found in live cells on the bronchial epithelium (93). The "inert" particles are removed chiefly via the transport of secretion in the bronchi, while a larger proportion of the cytotoxic particles end up in the lymph system. The entry to the lymph system is said by e.g. Macklin ([illegible]) and Cassaret (18) to lie in the small bronchioles.

According to these studies the cytopathogenic properties of the particles are of considerable importance for their penetration to the interstitial tissue and their transport in the lymph system. The presence of such particles may also affect the elimination of other "inert" particles. The cytopathogenic effect of a highly reactive substance such as quartz has for instance been shown by Klosterkötter & [illegible] (61) to lower the elimination and increase the penetration [illegible]

interstitial tissue of an "inert" substance in experiments with TiO_2 and corundum

LaBelle & Brieger (64) found that the addition of carbon particles accelerated the clearance of a low initial deposition of biologically active particles (uranium dioxide) from the rat lung The retention after 24 or 48 hours fell steadily as the amount of carbon particles deposited rose in the interval 0 005–1 5 mg A larger deposition of carbon particles reduced the rate of clearance again Bair & Hennecy (10) were unable to increase the clearance of inhaled $Ce^{144}O_2$ particles in the rat through the inhalation of carbon particles On the other hand the inhalation of graphite particles is reported by Friedberg & Polley (37) to block the elimination of quartz Furthermore LaBelle & Brieger (65) found that the rate of clearance was directly correlated to the number of free phagocytes in the rat lung From another study reported by Thomas (97) concerning rats subjected to prolonged exposure to uranium dioxide it seems that "the kinetics of loss from the lung are a function of the amount present at any time regardless of when it was deposited

The results of the above investigations indicate that the inhalation of a substance that affects one or several of the clearance mechanisms may protect the lungs against an injurious effect of another substance Studies have accordingly been made of the protective effect of various substances primarily against attack by SiO_2

The reticuloendothelial system (RES) has been stimulated experimentally with the aim of influencing clearance by Ferin (33) who demonstrated that pulmonary clearance in the rat is related to the functional state of the RES He also found that climatological factors affect pulmonary clearance which is retarded by prolonged exposure to cold

Accelerated clearance of SiO_2 is elicited by polyvinylpyridin N oxide (P 204) which appears to act through a protective effect on the phagocytes (61, 83 93) This agent causes SiO_2 particles to behave as an "inert" substance which may prevent penetration to the lymphatics

While the phagocytic cells i e the macrophages defend the organism from injurious effects of inhaled dust they are of still greater importance as a defence against bacterial infections The efficiency of this clearance mechanism is demonstrated by the fact that the lung in spite of the presence of bacteria in the air is sterile under normal conditions

The clearance of viable bacteria varies with their strain but is generally so efficient that a large proportion are removed from the lungs within a matter of hours apparently largely due to the activity of the macrophages (41)

The tension in the surface active film that covers the alveoli has been investigated in vitro by Mendenhall *et al* (69) Their work suggests that this surface active film assists the penetration of fluid from the alveoli to the bloodstream during exhalation and in the opposite direction during inhalation This would facilitate the gaseous exchange and also help to rid the alveoli from their own and foreign matter since the new surface active matter supplied to the alveoli at each respiratory cycle displaces the material supplied during the preceding cycle towards the ciliated epithelium

Another theory put forward by Gross (42) is that the film of fluid covering the alveoli varies in viscosity with the surface layer having the highest viscosity since this is where evaporation occurs At each inhalation this layer would lag behind the less viscous layer beneath it The more viscous layer would then be pushed upwards out of

the alveoli during subsequent expirations by newly formed viscous volumes.

A theory suggested by Antweiler (4) is that peripheral surface liquids are transported to the ciliated parts by a suction caused by the ciliary motility.

Tracheobronchial clearance mechanisms. The principle feature of the tracheobronchial clearance mechanism is a transportation of secretion that is promoted by the ciliary motility.

The cilia beat rapidly in an oral direction and recover more slowly. The number of beats per minute is approximately 1 000 in man (31), 1 100 in the rabbit (26) and 1 300 in the rat trachea (25). As a result of the ciliary movements the mass of secretion lying on top of the cells is transported in an oral direction towards the larynx. This transport may be effected by the tips of the cilia which lie in the more viscous layer of the secretion (52).

The cilia have been observed to beat in an axial direction in the calf trachea with deviations at the bronchial apertures and the vocal cords ([illegible]). It has also been found that particles are transported with secretion spirally in some cases ([illegible]) and axially (19) in others in the trachea. Furthermore the stream of secretion splits up at bronchial apertures where there may also be whirls in which particles are liable to remain for some time just as they are in "islands" of squamous epithelium ([illegible]).

The lack of cilia on the vocal cords and the anterior commissure of the larynx and their presence on the posterior commissure suggests that the secretion passes by the latter route.

The total cross section of the respiratory tract grows larger towards the alveoli and consequently the amount of secretion per unit length should increase in an oral direction; this relationship is offset, however, by an acceleration in the transport of secretion (52) in an oral direction.

Antweiler (4) has shown that different types of particles placed on the surface of tracheas from a number of different animals were transported at a uniform rate of about 15 mm/min and that this rate was not influenced by the character of the material, its size, weight or form. It therefore seems that the clearance achieved by ciliary activity is independent of particle size. Particle size does on the other hand affect deposition and consequently the amount eliminated may vary with this parameter. Some experiments have shown that the trachea is cleared of inhaled particles within 1 to 2 hours (95).

The motility of the cilia is affected by the composition of the secretion. An increased viscosity retards their movement and thereby reduces the beat frequency. The ciliary beat is also correlated to the temperature and humidity of the air. Further details concerning the morphology and physiology of the cilia have been published by Dalhamn (25), Sleigh (91) and Rivera (84).

The transport of secretion is influenced by other mechanisms as well. Dilatation and contraction during breathing may contribute to this transport. Although bronchial peristalsis has been demonstrated in some investigations (99), it is probably of secondary importance compared with the mucociliary transport mechanism.

The speed of the air flow may also contribute to the transport of particles out of the lung. Coughing may cause drops of secretion to be ejected from the respiratory tract. Forced inhalation on the other hand may result in drops of secretion with concomitant particles being displaced further down the respiratory tract.

Clearance via the lymphatic system

It is not clear where or by what mechanism or mechanisms particles enter the capillaries of the lymph system, but the translocation of particles to this system seems to be related to their toxicity (44 58 93)

As a rule the lymphatics receive only a small proportion of matter deposited in the lungs (96) though the amount varies considerably for different substances. The transportation of particles in this system is a slow process with a half life reckoned in years (60 62, 98) Large differences have been noted and the presence of cytopathogenic particles has been shown to increase the proportion up to 56 % compared with a normal figure of 1 % for the elimination of inert deposits via the lymphatic system (59) About 50 % of quartz deposited in the lungs finds its way to the lymphatics (58)

Lung clearance as a whole

Lung clearance as defined earlier can generally be described as the sum of at least two processes that occur at different rates Initially there is a rapid phase with a half life of hours This is followed by a slower phase (or phases) with a half life of weeks to months partly depending upon the nature of the material Ciliary activity is presumably the major factor during the initial phase while the subsequent phase (phases) probably involves chiefly the transport of matter from the deepest part of the lungs

As a rule lung clearance has been described only with respect to its course after the initial rapid phase Certain conclusions can however be drawn about total clearance from investigations concerning the slower phase or phases Inadequate data about the initial phase chiefly affect calculations of the level at which the slower phase starts in relation to the initial deposition

The rate of total clearance is dependent upon the physical-chemical properties of the particles (75), particularly their solubility in vivo Differences have been noted in the clearance of different substances, this has been reviewed by the Task Group on Lung Dynamics (96) The slower phase of clearance presents a typical pattern for the particles of a particular substance and may vary considerably between different substances.

In studies with heterodisperse aerosols of different sizes between 0.3 and 11 μm, however LaBelle & Brieger (14 64) found that the rate of clearance increased with the size of the particles they interpreted this as being due to the depth of deposition and not the particles solubility Harper & Morton (45) also found that bacterial spores tagged with an isotope were eliminated from the guinea pig more rapidly in the 4 and 6 μm sizes than in the 1 μm size. Similar results have been reported by Stockinger et al (92) for inhalation studies in the rat, using ca. 2 6 μm MMD and ca 0 45 μm MMD uranium oxide particles Moreover Albert & Arnett (1) conducted experimental studies with the inhalation of iron oxide powder tagged with Fe^{59} in man and found that the 3 4–4.3 μm particles were eliminated to a greater extent than the 1 4–2 3 μm particles during the first 2–4 hours

On the other hand, Bair & Willard (9) found that the smallest sizes were eliminated more rapidly than the larger sizes of 0 64, 3 3 and 4 3 μm MMD particles of $Pu^{239}O_2$ in the dog, and Thomas (97) found the same for 0.21 and 0 42 μm MMD particles of niobium95

In all the studies referred to above comparisons between the rates of clearance for different particle sizes in man or animals have not been studied simultaneously in the same subject.

In studies on the dog with various radioactive particles Morrow *et al* (73) stressed the importance of frequent observations during the first six hours of clearance in order to determine the initial retention in a satisfactory manner. Their studies showed the initial phase of clearance of submicronic particles to be a complex, variable phenomenon. To record it, however, they used a fixed unilateral location for the detector (over the thorax), which owing to the dependence of such measurements upon distance may explain the increase in activity measured during the first few hours. The measurements reported by Albert & Arnett (1) were subject to the same technical complications. Consequently these studies cannot be used to determine the half life of the initial phase of lung clearance. Moreover, apart from the last two reports, no investigations have provided sufficient data from the first hours after exposure for it to be possible to evaluate the initial phase.

Cember *et al* (21, 23) report that the soft beta radiations from S^{35} in radioactive barium sulfate particles do not affect the lung clearance in rats given up to 233 mc initially. They also found (20, 22) no injurious effect from a calculated total dose of 24 000 rep from particles deposited in the rat lung, indicating that irradiation up to this level from dispersed foci in the lung does not interfere with lung clearance. These and other investigations summarised by Hatch & Gross (47) show "that spatial separation of the radiation may reduce rather than increase the hazard from inhalation of relatively insoluble radioactive particles".

Data concerning lung clearance in man are based primarily upon observations of individuals accidentally exposed to radioactive aerosols. A few experimental studies have, however, been published (1, 74)[1]: the clearance is reported to be initially rapid and subsequently slower. The slow clearance phase for submicronic particles was very consistent in four individuals (74).

[1] While this monograph was at the printers, lung clearance studies using mono-disperse aerosols in man were reported by Albert *et al* (3a).

Earlier methods for studying lung clearance

Lung clearance has been studied with many different methods, most of which have involved experiments on animals sacrificed in groups at different intervals after exposure to an aerosol, the retention of which in the lungs was then determined by chemical, bacteriological or radioactive analysis. Such methods have the advantage that one can localise and make quantitative determinations of the amount of material retained in different parts of the lungs. Moreover, they are the only methods available at present for certain substances such as carbon and SiO_2. On the other hand, they have the disadvantage of requiring large series of animals for the experiments and one cannot study either intra- and interindividual variations or the effect of different substances on the course of clearance in the same individual on different occasions.

There has therefore been a need for methods by which the course of lung clearance can be studied for small initial deposits in one and the same animal; as a result, increasing attention has been paid in recent years to methods for external radiological measurement of aerosol retention.

Such methods were first used to follow the elimination of radioactive matter in individuals accidentally exposed to such aerosols or gases. This was done, for instance, by Marinelli *et al* (68) on six persons accidentally exposed to $RaSO_4$, using a scintillation detector surrounded by a lead collimator with a cone shaped top and a circular aperture. The projection of this aperture described a

circle 15 cm in diameter at a height of 10 cm above a mobile table placed over the collimator The patient lay supine on the table which could be moved to give a step wise scanning of the patient s body The total gamma activity in the lungs could then be estimated by integrating the areas under the scan curves and comparing these with the scan area for a known point source of Ra placed in a phantom

Apart from the investigations presented here experimental studies using profile scanning for the determination of lung clear ance have been made only by LaBelle *et al* (66) who took up such studies with the method introduced by Friberg & Holma (36) In studies on mice Bair *et al* (5 8 11) used a "scintillation counter designed to monitor either the whole mouse or any in crement along the length of the mouse This scanning unit had an unsatisfactory re solution and no attemps was made to eva luate lung clearance from the scan curves These were simply used to demonstrate the distribution of activity in the mouse at dif ferent times Stuart (94) also used pro- file scanning to demonstrate the distribution of activity from particles of $Pm_2{}^{147}O_3$ in haled by dogs

In other lung clearance studies both clin ical and experimental on animals and man a stationary detector or detectors has been placed over the thorax (1 3a, 34 73 74) In those cases in which repeated measure ments were made during the first hour or hours of clearance the results generally demonstrated an increase in activity probably because this measurement technique is af fected by changes in the distribution of the particles within the lungs As a rule this source of error has been avoided by limiting the study of clearance to its course after the initial rapid phase

These examples serve to illustrate one of the difficulties in following the entire course of clearance in an adequate manner and, in particular, the difficulty of evaluating the initial retention and hence the necessity of making frequent observations during the first hours after exposure

Since technical difficulties rule out the di rect determination in vivo of the pulmonary retention of particles tagged with alpha irradiating isotopes indirect methods have been developed for this purpose on the basis of faecal and urinary analyses (6)

Scope of the present investigation

From the literature it will be seen that much work has been done on the slower secondary phase of lung clearance whereas data con cerning the rapid initial phase are far from complete Although certain results suggest that lung clearance is affected by variations in particle size this parameter has not been investigated at all closely Variations in lung clearance on different occasions in the same individuals have only been investigated in one study concerning alveolar clearance of sub micron particles in six dogs by Gibb & Morrow (38) Furthermore there has been only one report (88) of experiments pe formed with the classical method on sacri ficed animals concerning the effect on lung clearance of gaseous air pollutants

The present monograph describes the de velopment of a profile scanning technique and reports the results of lung clearance studies on normal animals and animals ex posed to sulphur dioxide with particular reference to the influence of particle size and biological variations

Profile scanning was chosen as being superior to methods for external measure

ment that involve a fixed detector When using slit collimators profile scanning thus diminishes the problem of interference from activity in neighbouring organs in small animals, it is also relatively independent of displacement of the test aerosol in the lungs and it reproduces the distribution of activity in the animal s body

The method was first tried out on rabbits exposed to a heterodisperse aerosol and its suitability was tested by comparing these results with measurements of activity on isolated lungs

The method was subsequently used to study lung clearance after exposure to mono and di disperse aerosols (an octadecanol aerosol tagged with radioactive gold and polystyrene aerosols tagged with radioactive gold and radioactive scandium) These aerosols were produced by means of a modification of LaMer s condensation method [redacted] well as by a spinning disc technique

Only the first week or so of lung clearance could be studied with the profile scanning technique and consequently an open type of whole body counter was included in the equipment so that the clearance process could be studied over several months

PRODUCTION OF AEROSOLS

Choice of aerosol

The proper selection of a test aerosol for the study of lung clearance is dependent upon the purpose for which the study is intended and the method of measurement to be employed If the intention is to study the clearance of a particular substance this substance should be used for the test aerosol and this will limit the choice of measurement technique In the case of external measurements the test aerosol has to be tagged with some suitable radioactive isotope that does not affect the aerosol in other respects This rules out many substances including SiO_2 and carbon dust

In studies using animals the particles in the test aerosol should be tagged in a manner that will allow initial retentions in the microgram range to be measured Moreover since clearance varies with the size of the particles these should be monodisperse and/or have a reproducible size distribution

The choice of particle material and measurement technique is further limited in clearance studies with different particle sizes by the condition that these should as far as possible represent the same substance and form of particle the different sizes should also preferably be studied simultaneously in the same individual Obviously toxicological aspects as well as production techniques will govern the choice of materials for the particles

The choice of a suitable particle size will primarily depend upon the clearance mechanism or mechanisms to be studied as both the depth of deposition and phagocytosis are related to particle size

N octadecanol and polystyrene were selected for the production of the above mentioned particles by the condensation and spinning disc techniques With the exception of the preliminary study all the investigations by the author were conducted with monodisperse aerosols having particle sizes of 3 4 and 6 μm The aerosols used are described below together with the techniques for their production

Heterodisperse gelatin aerosols tagged with Au^{198}

In order to test the suitability of the profile scanning technique for recording activity retained in the lungs a preliminary study was conducted with a heterodisperse aerosol

Method and results

The heterodisperse particles were produced from a colloidal suspension (GCS 1) of radioactive metallic gold (Au^{198}) obtained from Amersham England and stabilised with gelatin It had a pH of 4—6 and the particles mean diameter was 3 5 nm The gold concentration was 10 mg per millilitre with a chemical purity greater than 99 % The specific activity was 10 mc per millilitre

The aerosol was obtained from a simple spray in which the airstream followed a circular path, the radii of which diminished successively Hence the largest particles were retained in the generator The aerosol particles obtained had a mean diameter of 1 5 μm $\sigma = 2\ 4$ as measured from photographs taken with a light microscope

Mono- and di-disperse aerosols tagged with Au^{195} and Sc^{46}

In principle one can produce monodisperse aerosols either by condensation or by dispersion of the substances in question (28, 40). The solution or suspension has been dispersed in principle by the formation of droplets using a mechanical influence in the form of gravitation, centrifugation or the application of electrostatic forces on the liquid. Since the mechanical systems require less sensitive apparatus, it is these which have been most used, particularly the spinning disc generators. These are most suitable for producing relatively large particles (1–100 μm), while smaller sizes (0.1–1 μm) are obtained better with the condensation generators, in which the substance is condensed onto condensation nuclei.

Condensation method for n-octadecanol particles

Monodisperse aerosols were first used in biological investigations by Wilson & LaMer (100) to study deposition in man. For this purpose they produced hygroscopic particles by means of a condensation method previously described by Sinclair & LaMer (90). The present author has employed monodisperse solid particles in the study of lung clearance since 1963 (53).

The condensation method used for the production of particles for lung clearance studies by the author was a modification of the technique described by Sinclair & LaMer (90) and has previously been published (29). Briefly, it works as follows.

Method

A generator produces condensation nuclei which are introduced together with a carrier gas into a heated glass boiler (Fig. 1) containing liquid coating material with saturated vapour above the liquid surface. The nuclei are conveyed by the carrier gas into another bulb with a higher temperature than the first one; here the vapour is superheated before passing out of the system through a chimney, where the vapour rapidly condenses on the nuclei, forming a monodisperse aerosol. This method is suitable for making monodisperse radioactive aerosols, provided the isotope can be introduced either in the nuclei or in the coating material. It proved possible to have the isotope in the condensation nucleus, but not in the coating material used in this study.

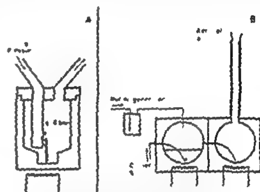


Fig. 1. Diagram of the aerosol generator used for the production of monodisperse aerosols by the condensation method. *A*: nuclei generator. *B*: La Mer device.

Au^{195} and Sc^{46} are isotopes which fulfill the necessary requirements, and n-octadecanol was chosen as a coating substance. It is only very slightly reactive, is insoluble in water and weak mineral acids, and has a melting point of 55°C, which means sufficient solidity at body temperature and a considerable vapour pressure at temperatures of 150–200°C.

It became apparent that a suitable way of introducing the nuclei into the LaMer system was to spray a solution of gold or scandium in the coating material into the first glass bulb.

The nuclei generator was made of brass and consisted of a cylinder with a conical bottom and a lid fitted with a pipe for the nitrogen. The capillary was attached to the pipe and its upper end was situated directly opposite the nitrogen outlet on the pipe; this outlet was a round hole 0.3 mm in diameter. The generator was heated to a temperature between 110° and 150°C and produced a

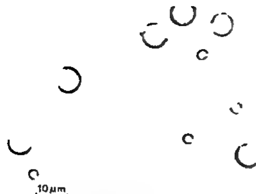


Fig 2 Photomicrograph of an n-octadecanol di disperse aerosol Mean particle sizes 3 1 and 7 5 µm

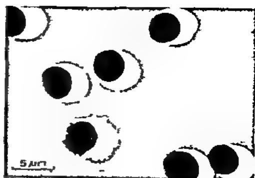


Fig 3 Electron micrograph of a monodisperse n octadecanol aerosol with a mean particle size of 4 5 µm

polydisperse aerosol in the first bulb when nitrogen under pressure was passed over one end of the capillary the outer end of which was immersed in the solution

Since both gold chloride and scandium chloride display a certain chemical instability the material in the nuclei generator could not be made more concentrated than 0 5 per cent of either substance At this concentration the solution is stable for at least one hour at a temperature of 150 C At higher concentrations there is sedimentation of reduced metallic gold or precipitation of scandium oxide in the nuclei generator Owing to further dilution of the nuclei material in the first glass bulb the maximum concentration of the radioactive material in the final aerosol is about 0 14 per cent corresponding to an activity of about 1 4 µc Au^{198}/mg aerosol

When the droplets are led into the first bulb at a higher temperature the n octadecanol evaporates and the gold or scandium material present forms condensation nuclei for the final aerosol

The size of the particles will depend in principle upon the relationship between the concentrations of the vapour and the condensation nuclei In practice these two factors depend upon several variables Thus variations can be made in the capillary diameter the temperature and gas pressure in the nuclei generator and the temperature and rate of flow of the carrier gas in the LaMer device

The solution to be used in the generator was prepared as follows 100 mg of $HAuCl_4 \cdot 3H_2O$ (or 169 mg of $ScCl_3$) was dissolved in 3 ml of warm ethanol and poured into 10 g of octadecanol at a temperature of 120°C. A homogeneous solution was formed and the ethanol evaporated.

Results

The particle size was varied from 1 to 10 µm and the standard deviation was of the order of 0 2 to 0 5 µm for the sizes used this gives an average coefficient of variation of 5 to 20 per cent

Fig 2 shows a mixture of two sizes of monodisperse particles 3 1 µm tagged with Au^{198} and 7 5 µm tagged with Sc^{46}

Studies of the particles with an electron microscope showed that they were either round or irregular Both kinds are shown in Fig 3

Discussion

This study showed that the condensation method can be used for the production of solid radioactive particles in a size range of interest for studies of lung clearance and that the radioactive tagging can be done with two isotopes (Au^{198} and Sc^{46}) having photopeaks that are clearly distinguishable by gamma spectrophotometry

The method has certain disadvantages

however when it comes to producing the highly active aerosols that are necessary for the study of low retentions In the first place there are only a few suitable substances that are not excessively reactive for biological purposes The number of substances is further limited by the need for a low vapour pres sure a melting point above the body tempe rature and the capacity to withstand heating in air or some other atmosphere (inert gas) to a sufficiently high temperature (gener ally above 100°C) for the saturation pressure to be high enough for the method to func tion properly

It is possible to tag the condensation nuc lei upon which condensation occurs as was done in the present study In this case how ever, good monodispersity requires that these nuclei are small in relation to the particles formed A reasonable activity per particle can still be achieved by using an isotope with a high activation cross section

The advantages of the condensation meth od include the absence of interference from particles of another size e g the satellite particles obtained with the spinning disc generator The disadvantages include the difficulty of obtaining a particular particle size as and when required This is due to a complex interaction between a large number of parameters such as the temperature and the rate of gas flow in the various containers of the apparatus as well as the temperature gradient and type of flow (laminar) in the condensation volume the rate of flow and a constant supply of condensation nuclei Keep ing all these factors constant places great demands upon the design and operation of the apparatus

Spinning disc method for polystyrene particles

The use of spinning disc generators to pro duce monodisperse radioactive aerosols for biological investigations was first reported by Albert *et al* (2) followed by the present author working with Kajland, Edfors & Friberg (56)

The following description concerns the production of the polystyrene particles that were used in the investigations presented in Chapter 7

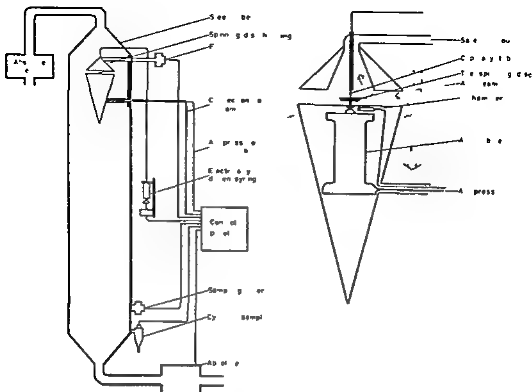
Method

The particles were produced with a spinning disc (Fig 4) which was a modification of the apparatus (56) used for the production of polymethylmetha crylate particles In principle the isotope material together with other particle material was dissolved or suspended in a solvent that was then fed at a constant rate to the centre of a rotating disc by means of an electrically driven syringe When the liquid was thrown off from the periphery two sizes of drops formed namely monodisperse main drops and smaller satellite drops The latter were arrested and evacuated by an opposing airstream in the slit through which the particles passed after leaving the dis This airstream was so regulated that for all practical purposes only the main drops were able to pass through the slit, thanks to their greater mass and hence their greater inertia These drops with an initial size of approx 60 μm, were then carried on a descending airstream while the solvent evaporated leaving material in the form of more or less spherical solid particles

The 6 μm particles were produced from a solu tion of 0 1 % polystyrene and 0 012–0 006 % gold chloride (Au^{198}) in xylene Just before the particles were produced the gold chloride was transformed into colloidal gold by bringing the solution rapidly to the boil

The 3 μm particles were produced from a solu tion of 0 01 % polystyrene and 0 005–0 001 % scandium acetylacetonate (Sc^{46}) in xylene The particles were produced in separate spinning-disc units driven at a speed of 21 000 r p m in both cases

The concentrations of colloidal gold and scand ium acetylacetonate in the finished particles were



F g 4 D agram of the sp nn ng d sc un t (see text)

approx mately 1 and 10 20 % respectively wh ch means that the 6 and 3 μm CMD particles mea sured approx mately 6 3 and 3 1 μm MMAD re spectively

Both types of part cles were collected on m lli pore f lters and then suspended in a known volume (2 cc) of a 50 per cent alcohol water solution or a pure water solut on by means of ultrasonic v bra t ons (90 kHz) for approx mately one m nute The concentrat on of the particles in th s solution was ascertained by counting the number of particles per cub c m ll metre in a Burker counting chamber After prof le scann ng (see Chapter 5) of a known amount of the suspens on plan metry of the prof le area obta ned was calculated as the number of prof le area un ts per 1000 part cles This measure was subsequently used to evaluate the s ze of the n tial retent ons in the rabb ts

The aerosol was regenerated by pass ng the par ticle suspens on through a simple spray up into an 18 l tre exposure chamber that was connected to the surround ng a r v a an absolute f lter (F g 18)

Results

The monodispersity as characterised by the coefficient of variation showed a range of 5 10 % for 3 and 6 μm CMD particles

The particles were stud ed in the optical as well as the electron microscope 3 μm par ticles tagged with Sc^{46} are shown in Fig 5 as seen through both microscopes while a mix ture of 6 and 3 μm polystyrene particles tagged with Au^{198} and Sc^{46} respectively is shown in Fig 6 as seen through the l ght microscope

F g 7 shows 6 μm polystyrene particles Two satell te particles are indicated by ar rows In all the experiments satellites amounted to less than 15 % by count of the main part cles

Fig 5 Electron micrograph (left, particles shadowed with silver) and photomicrograph (right) of monodisperse polystyrene aerosol without treatment with ultrasonic vibrations. Mean particle size 3.0 µm

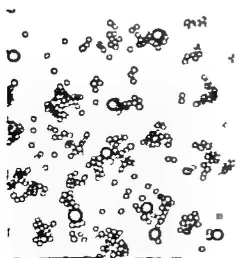


Fig 6 Photomicrograph of di-disperse polystyrene particles from two separate spinning disc filters before treatment with ultrasonic vibrations. Mean sizes [illegible] and 3 µm



Fig 7 Photomicrograph of monodisperse polystyrene particles after suspension in water by ultrasonic vibration. Mean size [illegible] µm. Two satellites are indicated by arrows

Discussion

As demonstrated by these and Albert *et al.*'s experiments (2, 3) the spinning disc generator can be used with suspensions or solutions of solid substances in fluids to produce monodisperse particles with a high specific radioactivity that are suitable for the study of lung clearance. The size of the primary drops can be varied between 20 and 100 μm chiefly by adjusting the speed of the rotating disc. Final particle sizes down to approximately 1.0 μm can be obtained by varying the dilution of the dissolved material in the fluid. The purity of the base fluid is most important here since this determines the maximum dilution and consequently the size of the smallest particles that can be obtained.

This method can be used to produce final particles with a high content of the isotope material and hence a high degree of radioactivity. A variety of materials are available for the production of particles since the apparatus can be used for all liquids having a low viscosity as well as for suspensions and solutions in these. The chief disadvantage is the formation of satellite drops.

In order to avoid satellite particles it is necessary for the fluid to be introduced at a low rate of flow onto the disc, and to have the airstream that evacuates satellite drops properly adjusted and evenly distributed around the periphery. It is also important that the capillary supplying the fluid is placed exactly over the centre of the disc, as otherwise the rotation produces a disturbance in the form of drops on the capillary. The surface treatment of the disc is also important as is the quality of the turbine that drives the disc particularly at high speeds.

CHAPTER 3

TRANSLOCATION, SOLUBILITY, RETENTION AND PHAGOCYTOSIS OF THE AEROSOL PARTICLES

The solubility retention and propensity to phagocytose displayed by the aerosol particles may affect their translocation as well as their clearance in general Although the following experiments cannot claim to be exhaustive they provide sufficient data to indicate the method s validity besides throwing some light on the differences in deposition and phagocytosis between 6 and 3 μm particles

The solubility and translocation of the gelatin particles are dealt with first in conjunction with the methodological tests of the profile scanning unit Data concerning leaching translocation retention and phagocytosis are then reported from experiments involving the use of the 6 and 3 μm particles The particle characteristics of the octadecanol aerosol are considered only briefly because this aerosol was not utilised in the experiments reported here

Gelatin particles tagged with Au^{198}

Method

Male rabbits were sacrificed in groups of four by giving pentobarbital intravenously at different intervals (0 25 0 5 1 2 4 8 and 16 hours) after exposure via a tracheal tube to the heterodisperse gelatin particles tagged with colloidal gold (Au^{198}) The following organs were then immediately removed wet ashed in concentrated HNO_3 evaporated to 10 ml and measured in a well counter for their activity liver spleen kidney about 3 cm of the right femur a piece of the parietal bone oesophagus trachea stomach intestines blood urine and lungs

The activity in the gastrointestinal tract was found to be so considerable when measured with a conventional scaler technique (using a scintillation detector in a simple lead shield) that it was not subject to further analysis

Results

In no case did the activity in the liver spleen adrenals blood urine or other organs exceed 0 1 % of the activity in the lung

The activities in the oesophagus and trachea expressed as a percentage of the lung activity are shown in Table 1 The activity in the oesophagus was found to be 0 1 % or less in approximately half the cases values exceeding 1 % were found in only 5 cases The values for the trachea varied considerably but in only 2 cases did they exceed 10 % of the activity in the lung

Discussion

The negligible activity observed in isolated organs after resorption had occurred to other organs means that one can ignore the direct translocation to these from the lungs Thus in specific organs—in particular the liver where it is known that gold supplied parenterally can accumulate—no gold could be demonstrated In electron microscopic studies on the rat Gieseking (39) showed that colloidal gold particles injected intratracheally have an intracellular location in the alveolar cytoplasm after 3 hours where they remain for a certain unspecified

TABLE 1 Activity in the eosophagus and trachea in percent of the activity in the lung at different times after the end of exposure

	Time after the end of exposure in hours						
	¼	½	1	2	4	8	16
Activity in eosophagus in percent of lung activity	<0 1	0 1	0 3	0 3	<0 1	<0 1	<0 1
	<0 1	3 3	0 2	1 2	<0 1	0 1	<0 1
	2 4	0 2	<0 1	0 7	0 4	0 5	0.2
	<0 1	0 7	1 1	6 3	0 2	0 1	<0 1
Activity in trachea in percent of lung activity	3 5	13 1	1 9	1 8	0 7	2 5	0 4
	0 9	2 0	10 3	0 5	0 2	3 4	<0 1
	1 3	4 6	5 2	0 3	<0 1	0 2	3 5
	1 7	1 0	1.5	0 9	0.2	0 1	0 7

time Moreover Meneely *et al* (70) have shown that after transtracheal instillation of radioactive gold colloid in the lung of dogs little if any of the colloid enters the blood stream

During profile scanning (see Chapter 5) a scan peak was observed above the stomach quite soon after exposure and later above the intestines One can therefore assume that a decline in the "lung peak" is essentially due to the inhaled gold being cleared from the respiratory organs via the trachea

N octadecanol particles tagged with Au^{199}

The leaching of gold (Au^{198}) in various media (water weak acids rabbit serum) was investigated and it was found that the tagging isotope was relatively firmly embedded in the octadecanol particles Consequently there should be no considerable leakage of the isotope when the particles are in lung tissue Certain results have already been published (29 56) and no further details will be reported here as clearance studies with octadecanol particles were not included in the present investigation

Polystyrene particles tagged with Au^{199} or Sc^{46}

Polystyrene was chosen for the production of mono and di-disperse particles by the spinning disc method because of its solubility in volatile solvents such as aromatic hydrocarbons (e g toluene and xylene) as well as its insolubility in water weak acids aqueous alkalies and alcohol Moreover it has been reported that polystyrene particles are "not toxic and non antigenic (so far as present procedures can determine) and there is no indication that they are metabolised or cause histologic or cellular damage (86 87)

In the lung clearance studies (Chapter 7) the polystyrene particles were regenerated to an aerosol from a 30 % alcohol water solution Alcohol was included because this made it easier to obtain a suspension of the particles However the 3 μm particles used in these experiments were tagged with Sc^{46} in scandium acetylacetonate which is soluble in alcohol Leaching of this isotope from the particles was therefore investigated and the same was also done in the case of the 6 μm colloidal gold particles tagged with Au^{198} Furthermore the translocation of these tagging isotopes from particles deposited in the

lungs was investigated as was phagocytosis of these particles by lung macrophages in vitro

Leaching

The study was made on 3 µm particles in 10 and 16 % concentrations of scandium acetyl acetate with a specific activity of 760 µc/mg Sc. The particles produced by the spinning disc technique were collected on duralon millipore filters from which they were transferred to small test tubes with a marten hair brush together with 2 cc 50 % alcohol water solution from a pipette The test tubes were then measured in a multichannel gamma spectrometer (AB Atomenergi Studsvik) Before and after remaining in this alcohol solution for various periods up to 2 hours the suspension was treated with ultra sound before being rinsed with 20 cc of the same alcohol solution in a funnel containing a fibre glass absolute filter underneath a cellulose millipore filter (pore size 0 8 µm) Weak suction by water was used to assist filtration The total eluate was then measured in the same apparatus as above and its activity in relation to the activity in the filtered volume was taken as a measure of the percentage leaching of Sc^{46} from the particles Five experiments gave an average leaching of 1 6 % with a maximum of 1 9 and a minimum of 1 3 %

In other similar experiments leaching of less than 1 % has been obtained for both Au^{198} and Sc^{46} using the same concentration of alcohol as above as well as aqua font and 0 1 N HCl

Albert *et al* (3) have also performed leaching studies on lucite particles tagged with acetylacetonate of Cr^{51} Fe^{59} Zr^{95} and Nb^{95} They found negligible leaching of the isotopes in a physiological solution during a period of several weeks

Retention and translocation

This study was done to evaluate the relation ship between retention of 6 and 3 µm CMD (6 3 and 3 1 µm MMAD) particles at different levels in the rabbit lung as well as any translocation of the tagging isotopes in these particles

Method

The investigations were performed on 15 male rabbits with a normal rectal temperature weighing about 3 kg Each of the animals was exposed simultaneously to monodisperse 3 and 6 µm polystyrene particles tagged with Sc^{46} and Au^{198} respectively During the exposure which lasted about 10 minutes and occurred via a tracheal tube under anaesthesia (Nembutal Abbot approximately 3 mg/kg bodyweight) the animal was placed in a whole body respirator This made it possible to adjust the respiratory volume individually to roughly 25 cc with a constant rate of about 20 breaths per minute

The aerosol which the animal inhaled from a simple exposure chamber (Fig 18) was produced by spraying a water suspension containing the radioactive 3 and 6 µm particles

In order to obtain approximately the same deposition in the lungs of the different animals, the accumulated activity in the lungs was checked with a scintillation detector coupled to a ratemeter placed under the thorax (Fig 18)

Each animal was subjected to profile scanning in apparatus no 4 (see Chapter 5) as soon as possible after the end of exposure The animals were divided into three groups of five rabbits each The first group (rabbits 01–05) were sacrificed immediately after the profile scanning and the other two (rabbits 21–25 and 721–725) [illegible] and 72 hours later respectively immediately after a second profile scanning Various organs were then removed the lungs from the animals in the 1st and 3rd groups being taken without allowing them to collapse These lungs were dehydrated (Fig 8) by keeping them inflated to roughly their normal size with paraformaldehyde vapour and compressed air using a heating lamp to accelerate the process A parallellepiped (Fig 9) was cut from the lower lobe of each lung in such a way that a secondary bronchus appeared at one end while the

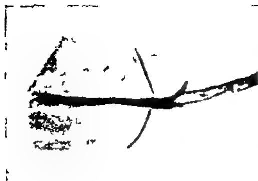


Fig 8 Formalin fixed air dried lung of rabbit (see text)



Fig 9 Parallellepiped cut out of the lower lobe of the right lung shown in Fig ▮ The specimen has been cut to show that its axis runs parallel to the axis of the secondary bronchus (to the right in the figure)

longitudinal axis ran in roughly the same direction ▮ this bronchus The outer end thus contained the alveolar region facing the inferior margin One such specimen was taken from the left inferior lobe and one from the right in each of the animals in these two groups Each specimen was divided into four pieces of approximately the same size (Fig 10) and the activity from Au^{198} and Sc^{46} respectively was measured in each piece using a well type 3" × 3" NaI crystal coupled to a 512 channel pulse level analyser The mean weight of the specimens was found to be 77 mg $\sigma = 45$

In order to reduce variations arising from differences in the weights of the specimens and the initial depositions in the lungs the calculated weights of the particle sizes for each piece in a specimen were divided both by the weight of the specimen and by the size of the initial deposition in the lung The measures of activity were converted into weights of particle material by assessing the activity in known amounts of the same particle material with activity measurement and profile scanning of the same sample

After these measurements the specimens were returned to their parent lungs and these as well as the trachea (from about 1 cm cranial of the bifurcation to just below the cricoid cartilage) oesophagus liver spleen and kidneys (the last three together) were homogenised in 20–30 cc of equal parts of gently heated HNO_3 and H_2SO_4 The samples were diluted with water to 50 cc and stored in 500 cc round bottomed flasks Urine was collected from the 3rd group of animals which had been kept in metabolism cages and was divided into portions of 50 cc in similar vessels The activity in these samples was measured with a 3" × 3" NaI



Fig 10 The parallellepiped in Fig 9 cut into four pieces to show how the different specimens for the retention studies were obtained (see text)

crystal in the same apparatus ▮ the pieces of lung mentioned above (the measurements were made by AB Atomenergi Stockholm)

The activity in the trachea and oesophagus was calculated as a percentage of that in the lung The correlation between the activity measured in the isolated and homogenised lungs and the estimate from the lung scan fraction on the profile curves is reported in Chapter 6 The results given below concern the retention study mentioned above and the translocation of the tagging isotopes to the spleen liver and kidneys in relation to the amounts of each isotope initially deposited as well as the percentage of the isotopes in the trachea and oesophagus in relation ▮ the activity in the lungs at the same time

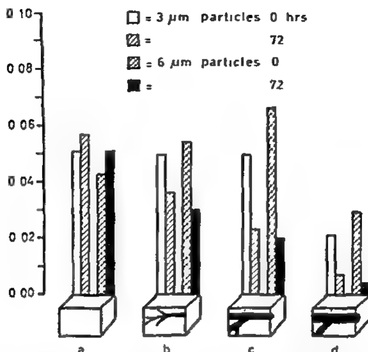


Fig 11 The amount of monodisperse 3 and 6 µm polystyrene particles in the different lung specimens at 0 and 72 hours after inhalation The vertical scale gives the amount of particles in the specimen in µg per 100 µg of tissue divided by the value of initial retention in the lung of these particles *a* — *d* refer to the specimens cut out of the lungs *a* referring to the most peripheral specimen and *d* to the one containing the secondary bronchus (see text and Fig 10)

Results

The mean weights of 6 and 3 µm particles in the specimens cut out of the lungs from two groups of five rabbits are shown in the bar diagram in Fig 11 (each specimen from one lower lobe has been added to its pair from the other lung)

It will be seen that the 6 µm particles have a maximum in a region just peripheral to the secondary bronchi while the 3 µm particles are deposited more in the alveolar regions having an approximately equal rentention in each of the three most peripheral specimens After 72 hours there is little change in the most peripheral piece *a* while both sizes display a reduction that increases towards the bronchi being significant in pieces *c* and *d* (t test $p < 0.02$ and < 0.01) This change is more pronounced for the larger particles both in terms of weight and on a percentage basis The latter relationship is illustrated in Fig 12 which shows that the percentage reduction from 0 to 72 hours increases towards the bronchi and is greater for 6 than for 3 µm particles

Fig 13 gives the ratios between the amounts of 6 µm and 3 µm particles at 0 and 72 hours in the specimens and in the

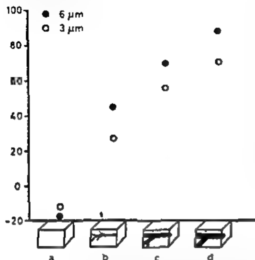


Fig 12 The percentage amount of 6 and 3 µm *polystyrene particles eliminated from the different* lung specimens (see Fig 11 and text) in 72 hours after inhalation The reduction is greater for the 6 µm particles compared with the 3 µm particles except in the most peripheral speci men both sizes of particle are reduced more in specimens containing larger bronchi

trachea in relation to the corresponding initial ratios in the lungs as assessed by pro file scanning It will be seen that the dif ferences between the ratios at [redacted] and 72 hours grow larger towards the trachea The figure also demonstrates the predominance of 6 µm particles in the bronchial pieces and in the trachea at 0 hours

Fig 14 shows the retention in the lungs at different intervals after exposure com pared with the initial values calculated from the scan curves It was found that the larger particles were cleared from the lungs more rapidly than the smaller ones

In the case of the trachea the activity measurements (cf Table 2) show that the 6 µm particles were initially deposited there to a greater extent than the 3 µm particles This is also indicated by the scan curves Thus in Fig 15 which shows the distribu tion of the initial deposition of the two particles in rabbit no 724 tracheal activity was demonstrated for the 6 µm particles tagged with Au^{198} but not for the 3 µm particles tagged with Sc^{46} Table [redacted] gives the activities in the tracea [redacted] percentages of the activity in the lung for both isotopes These data show that the first measurements for Au^{198} and Sc^{46} amount to maxima of 73 and 22 % respectively At 2 hours the corresponding figures are 22 and 12 %[1] and after 72 hours 0 4 and 0 14 %

The radioactivity of the tagging isotopes in the liver spleen and kidneys as well as in urine is shown in Table 2 It will be seen that only negligible quantities (averaging 0 8 and 0 2 % of Au^{198} and Sc^{46} respec tively of the amounts initially deposited in the lungs) could be found in these organs and in the total urine collected during 72 hours It will also be seen that the activities in the oesophagus were never more than 0 8 and 0 1 % for Au^{198} and Sc^{46} respectively

Discussion

These experiments show that the 6 µm par ticles are deposited more in the bronchial sections compared with the 3 µm particles Moreover the larger particles disappeared more rapidly than the smaller ones from the trachea and bronchi as well as from the lungs as a whole

These results cannot be directly compared with those from other studies chiefly owing to the considerable differences in exposure

[1] The data for rabbit 21 (33 4 and 45 1 %) have been excluded because of a technical error during dissection (cf p 65)

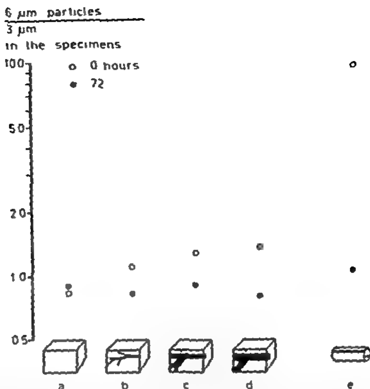


Fig 13 The ratio between the amount of 6 and 3 μm particles in relation to the initial retention in the lung (means for specimens from right and left lung in 5 rabbits) The amount of 6 μm particles in relation to 3 μm particles dominates in the bronchial pieces and still more so in the trachea The letters *a* — *d* are as for Fig 11 *e* refers to the trachea

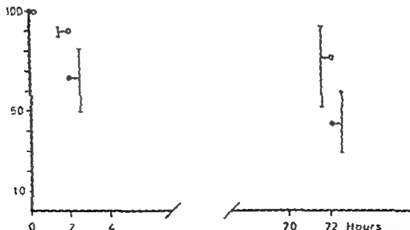


Fig 14 The amount of 3 and 6 μm polystyrene particles at different times in the lungs of rabbits as a percentage of the initial value The 6 μm particles are eliminated to a greater extent than the 3 μm particles

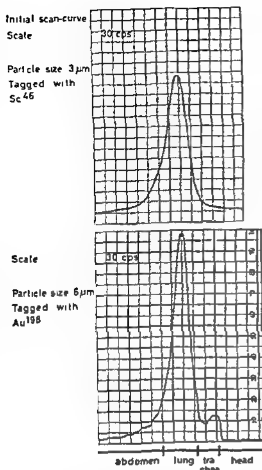


Fig 15 The scan curves for rabbit no 724 in the study mentioned in the text The curves were re corded immediately after exposure to a di-disperse 6 and 3 µm polystyrene aerosol via a tracheal tube There is a laryngo tracheal deposition of the 6 µm but not the 3 µm particles

and measurement techniques The picture of deposition presented here does in fact largely correspond with that reported by Palm *et al* (79) and Taplin *et al* (95), who found that the larger particles were deposited more than the smaller ones in the upper part of the respiratory tract in experimental animals

The more rapid elimination in the higher reaches of the respiratory tract is also in line with Taplin *et al's* (95) report that P32 tagged *B subtilis* spores (0 5—1 5 µm) are eliminated from the rabbit trachea in one to two hours but remain in the lung paren chyma for at least seventy two hours

The present results concerning the size of the retention in the trachea and lungs at 0 hours may have been influenced to some extent by the displacement of the secretion containing the particles towards the trachea during the interval between the sacrifice of the animal and the cessation of ciliary activi ty with the addition of fixative Such a dis placement would mainly affect the assess ment of activities in trachea and lung par ticularly the initial values This circumstance may explain the results of the activity mea surements of these organs Comparing the scan curves taken just before the animals (01—05) were sacrificed with the sub sequent activity measurements it will be found that the activity in the trachea was considerably higher on the latter occasion Moreover a corresponding reduction of the lung activities in these animals was revealed by the correlation study (cf Fig 46) This displacement of activity from the lung to the trachea could have been partly avoided by clamping the trachea at the bifurcation during the dissection instead of just below the cricoid cartilage [redacted] was done here The present procedure was adopted to facilitate preparation of the lungs for the special de hydration technique This technique which

TABLE 2 *Retention and translocation of 3 and 6 µm CMD polystyrene particles tagged with Au^{198} and Sc^{46}*

Rabbit no	Initial retention µg 6 µm	Initial retention µg 3 µm	Initial retention µc Au^{198}	Initial retention µc Sc^{46}	Post exposure time (hrs)	Radioactivity in organs in percent of initial activity in lung: Lung Au^{198}	Lung Sc^{46}	Liver, spleen and kidneys Au^{198}	Liver, spleen and kidneys Sc^{46}	Urine 0—72 hrs sample Au^{198}	Urine 0—72 hrs sample Sc^{46}	Radioactivity in organs in percent of activity in lung at the same time: Trachea Au^{198}	Trachea Sc^{46}	Oesophagus Au^{198}	Oesophagus Sc^{46}
01	5.7	2.4	11	10	0							72.6	2.7	0.30	0.01
02	4.4	1.2	9	5	0							13.3	5.6	0.09	0.12
03	6.5	1.5	15	6.5	0							68.7	21.7	0.09	0.02
04	6.5	1.3	9	5	0							70.3	9.4	0.05	0.02
05	7.6	1.3	12	5.5	0							36.7	10.4	0.03	0.03
Mean	*6.0*	*1.5*	*10.8*	*6.4*								*58.4*	*10.0*	*0.11*	*0.04*
21	3.8	1.5	11	6	2	58.1	92.5					334.0[1]	45.1[1]	0.36	0.01
22	3.8	2.2	10	9	2	71.8	91.2					0.3	0.5	0.01	0.06
23	2.7	2.0	6	8	2	81.9	92.3					22.0	11.8	0.15	0.05
24	4.5	1.9	11	8	2	50.1	90.0					27.1	6.8	0.02	0.01
25	6.2	1.1	17	15	2	72.7	87.8					5.2	3.3	0.02	0.12
Mean	*4.3*	*1.7*	*11.2*	*7.1*		*66.9*	*90.4*					*12.4*	*3.6*	*0.10*	*0.06*
721	3.6	2.7	11	11	72	60.0	92.6	0.54	0.11	0.26	0.01	0.02	0.03	0.11	0.01
722	4.6	3.1	9	13	72	17.6	83.3	1.30	0.22	0.61	0.04	0.12	0.11	0.79	0.01
723	5.5	3.9	10	16	72	15.3	52.8	1.16	0.16	0.30	0.01	0.22	0.11	0.02	0.004
724	17.5	1.8	23	7	72	35.0	87.8	0.31	0.16	0.12	0.04	0.12	0.11	0.002	0.01
725	12.2	5.3	18	11	72	29.6	68.4	0.79	0.11	0.21	0.02	0.39	0.13	0.02	0.01
Mean	*8.1*	*3.0*	*14.2*	*12.2*		*43.5*	*77.0*	*0.92*	*0.16*	*0.30*	*0.02*	*0.23*	*0.10*	*0.19*	*0.01*

[1] These values which were subject to technical error during dissection (see p. 65) are not included in the mean

has not previously been used for retention studies carries the advantage that any part of the lung can be excised and examined for the material retained in it

The translocation studies indicate that the tagging isotopes were relatively securely embedded in the particles This was also demonstrated by the leaching experiments reported earlier in this chapter The liver kidneys and spleen were chosen as representative organs together with the urine since it is in these sites that the translocation of soluble radioactive substances deposited in the lungs has been reported to occur by several authors

The negligible degree of translocation of the lung radioactivity from these tagged polystyrene particles may be taken to indicate that these have been cleared from the lungs via the bronchi and trachea This means that recordings of the residual retention of such particles in the lungs at different times can be used as a measure of their clearance from the lungs

The activity that happens to be passing through the oesophagus during a recording of the activity in the lungs naturally represents a source of error in these measurements However in the present studies as well as those with heterodisperse gelatin particles tagged with colloidal Au^{198} the activity demonstrated in the oesophagus was of no practical significance On the other hand this is no indication of the amount of activity that is swallowed and thus passes the lung region but simply of the activity that remains after the quantities swallowed have passed by By using the profile scanning technique it is possible to determine the time when such quantities pass the lungs In some cases this can be detected from the resultant interference on the scan curves before and after such transport has taken place

Phagocytosis of polystyrene particles in vitro

Although the part played by phagocytosis in lung clearance is still being debated it has been established that different particle materials and particle sizes are phagocytosed to different degrees and that phagocytosed particles are to be found both in the walls of the respiratory tract and in the interstitial tissue lymph ducts and lymph glands Having undergone phagocytosis the particles may be affected so that for instance their solubility is altered (18)

Various principles and methods have been applied to the study of phagocytosis in vitro (76 101) The technique introduced by Myrvik *et al* (77) and its several modifications have been most used in recent years One such modification was employed in the following investigations into the phagocytosis of different sizes of polystyrene particles by lung macrophages

Method

Phagocytes (macrophages) were collected from rabbit lungs with a technique described by Myrvik *et al* (77) A rabbit was sacrificed by injecting air into a ear vein The lung was removed with the trachea clamped in order to prevent blood entering the specimen and was rinsed with a warm physiological NaCl solution (37°C) After the heart had been removed the lung was cleaned with gauze and about 40 ml Hank s saline solution (37°C) was injected into each of the main bronchi to inflate the lobes The trachea was unclamped and the lung was gently massaged The liquid was then collected in a large centrifuge tube and the process with Hank s solution was repeated The two tubes were then centrifuged for 20 min at 1 500 r p m The liquid was decanted and the cell mass re suspensed in 3 ml physiological NaCl solution before repeating the centrifugation The liquid was again decanted and the cell mass re suspended in 2 ml of Hank s solution The viability of the cells was then checked by vital staining (equal parts of cell suspension and 0 1 % eosin solution)

The suspension was found to contain a total of about 12–16 million macrophages. For the studies of phagocytosis 0.15 cc of the cell suspension was pipetted onto a number of scratched circular cover glasses (2.8 sq. mm) and left to settle for 40 minutes, after which the glasses were decanted and rinsed in Hank's solution. The cover glasses were then placed in small Petri dishes (inner diameter 18 mm, depth 8 mm) and 0.5 cc of a particle suspension was added consisting of Parker 199 solution, 25 % autologous rabbit serum, penicillin (100 i.u./ml) and monodisperse polystyrene particles (pure untagged or tagged with inactive or active material, i.e. Au^{198} and Sc^{46}). The Petri dishes were covered with a lid and stored in a thermostat at 37°C.

The total number of cells, the number of these that had phagocytosed particles and the number of particles that had undergone phagocytosis were all calculated for a particular unit of area (the visible field in direct observations and the corresponding 0.52 sq. mm. of the cover glass on photographs taken with a light microscope and enlarged 320 times). A sufficient number of fields were counted in this way to give about 700 cells for the assessment of the degree of phagocytosis, i.e. the number performing phagocytosis in relation to the total number of cells on the surface in question. The ratio of cells to particles averaged 1 : 4 (see Table 1 in the Appendix).

In order to guard against an erroneous assessment of the number of phagocytosed particles, a control series equivalent to the above was produced using cells killed with formalin. It was found that not more than 1 % of the total number of cells had particles that coincided with their projection.

Four series of experiments (A, B, C and D) were performed with polystyrene particles. Table 3 gives the code numbers used in the studies of phagocytosis for the various combinations of particle materials and sizes. The 1.5 μm particles were satellites of the 6 μm particles and were deliberately included in these studies in order to compare the degree of phagocytosis of the different sizes of particles. The monodispersity of both the 3 and 6 μm particles showed a coefficient of variation of < 10 %. The particles tagged with Au^{198} or Sc^{46} had an average specific activity of approx. 0.1—0.5 and 0.5 μc/100 000 particles respectively. In the case of the particles tagged with Au^{198}, the activity came from both the 6 μm particles and their satellites but the calculation was based on the 6 μm particles only.

The phagocytosis of carbon particles (approximately 0.5—2.5 μm) was studied (series F) in the manner described above in order to compare the phagocytosis of these and the polystyrene particles.

Results

Results for the various series are given in the Appendix (Table 1) together with the concentrations of the particles and phagocytes. About 95 % of the cells were viable at the start of the experiments and this figure had generally dropped only about 1 or 2 % by the end.

The degree of phagocytosis in the different series after 1 1/2 hours is shown in Fig. 16 in terms of the number of cells performing phagocytosis as a percentage of the total number of cells per square millimeter. The degree of phagocytosis after 3 hours was practically the same. It will be seen that the highest degree of phagocytosis involved the smallest polystyrene particles (approximately 20 %) and that the lowest level of phagocytosis was displayed by the largest of the tagged particles (6 μm). No pronounced differences could be found in the degree of phagocytosis between the different sizes of untagged particles. A light microscopic photograph (Fig. 17) illustrates the predominance of 1.5 μm particles in the macrophages compared with 6 μm particles.

Owing to the small number of independent experiments with the different sizes of particles, the results have not been subjected to statistical analysis.

The result of the separate study concerning phagocytosis of 0.5–2.5 μm carbon particles is also shown in Fig. 16. There is a marked difference in the degree of phagocytosis between these particles and those of polystyrene.

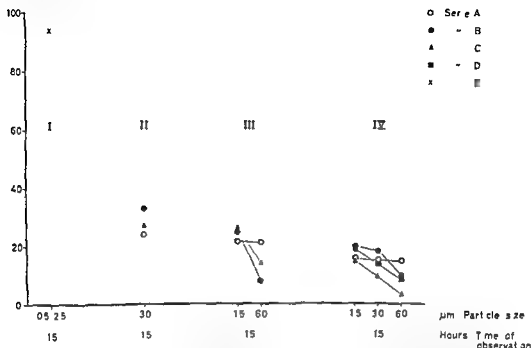


Fig 16 The degree of phagocytosis of 1 5 3 and 6 µm polystyrene particles by rabbit lung macrophages determined as the percentage of macrophages that have taken up one or more particles in vitro The code for the symbols is given in Table 3

TABLE 3 Code for particle sizes and combinations in the study of phagocytosis

A II = 3 µm	polystyrene particles untagged
A III = 1 5 and 6 µm	polystyrene particles untagged
A IV = 1 5 3 and 6 µm	polystyrene particles untagged
B II = 3 µm	polystyrene particles tagged with Sc
B III = 1 5 and 6 µm	polystyrene particles tagged with Au
B IV = 3 µm	polystyrene particles tagged with Sc
B IV = 1 5 and 6 µm	polystyrene particles tagged with Au
C II = 3 µm	polystyrene particles tagged with Sc^{46}
C III = 1 5 and 6 µm	polystyrene particles tagged with Au^{198}
C IV = 3 µm	polystyrene particles tagged with Sc^{46}
C IV = 1 5 and 6 µm	polystyrene particles tagged with Au^{198}
D IV = 1 5 3 and 6 µm	polystyrene particles tagged with Sc^{46}
E I = 0 5 2 5 µm	carbon particles



Fig 17 Photomicrograph of phagocytosis of 6 and 1.5 µm polystyrene particles by rabbit lung macrophages. Particles can be seen intra and intercellularly. The machophages contain more smaller than larger particles

Discussion

These results should be assessed in the light of the method employed. In vitro studies of phagocytosis on glass receptacles clearly do not represent conditions in vivo. From a review of methods for such studies (101) it appears that phagocytosis is promoted if allowed to proceed on a base of freshly excised tissue (e.g. lung specimens fixed with formalin) or on an inert surface such as that of moistened filter paper: the phagocytes can then ingest the particles by trapping them against the walls.

Scratched glass (the bottoms of small glass bowls) was used as a base for the phagocytes in the present method. According to Myrvik *et al* (77) about 16 million cells can be obtained from the lungs with this technique, a range that embraces the total of 12–16 million cells obtained in the present study. Acording to Myrvik's investigations, the great majority of these cells are macrophages: less than 0.1 % are polymorphonuclear cells and still fewer are small lymphocytes. A predominance of macrophages was also found in the present series.

The results cannot be compared directly with those obtained by other authors since the latter have been derived from other types of phagocytes and the methods have also varied.

There are, however, indications that the rate of phagocytosis is higher for the larger polystyrene latex particles of different sizes in the range 0.514–1.171 µm, as shown by Schoenberg *et al* (87). After these particles had been introduced intravenously into rabbits the larger ones disappeared more rapidly from the bloodstream, an effect that was ascribed to phagocytosis in the reticuloendothelial system. Krenis & Strauss (63) also found signs that phagocytosis declines with the size of polystyrene latex particles in the range 0.264–1.171 µm, in that oxygen uptake increased with increasing particle size for phagocytic leucocytes from human blood. No increment was found in leucocytic respiration for the smaller particles and this was interpreted as indicating that there is little or no phagocytosis of the smallest particles. Schoenberg *et al* (87) consider, however, that since phagocytosis involves the expenditure of energy, the smaller particles may enter the cells more easily, but they are also ejected at a more rapid rate because the extrusion of particles from the phagocytic cells also requires energy; these circumstances may lead to the apparent observation of a greater phagocytosis of larger particles.

Fenn (32) found that leucocytes from rat peritoneum ingested carbon particles of 4–5 µm more readily than those of 2—3 µm. He interpreted this as being due to the phagocytic cells having a better chance of coming into contact with the larger particles. This is unquestionably correct but there must exist a limit above which phagocytosis declines for purely mechanical reasons; hence there should be a certain optimal size of particle

that gives a maximal rate of phagocytosis This size will probably be displaced upwards for particle materials that are "susceptible" to the phagocytes This is also suggested by Mudd *et al's* (76) observation that small emulsion drops are readily and completely ingested by macrophages whereas on larger drops the macrophages spread to positions determined by the balance between surface forces and their own resistance to deformation Jötten & Marwyck (55) also suggest "that dust particles must not exceed 8 μm as those larger than this are poorly phagocytized or perhaps not at all"

In the light of these reports the present results may be said to indicate that optimal phagocytosis of the tagged polystyrene particles should occur at a size below or around 1 5 μm Further investigations are required however, as well as studies on smaller sizes of particle before this figure can be established

A comparison between the phagocytosis of carbon and polystyrene particles shows that the former stimulate phagocytosis to a considerably greater extend than the latter

The difference in the degree of phagocytosis between the tagged and untagged 6 μm particles has not been further investigated It should however be noted that not many experiments were conducted with untagged particles It is also worth mentioning that the lower phagocytosis of large particles was particularly pronounced if they were tagged with gold The same difference between sizes of particles was found in the experiments in which all sizes of particle were tagged with scandium



Fig 17 Photomicrograph of phagocytosis of 6 and 1 5 μm polystyrene particles by rabbit lung macrophages Particles can be seen intra and inter cellularly The macrophages contain more smaller than larger particles

Discussion

These results should be assessed in the light of the method employed In vitro studies of phagocytosis on glass receptacles clearly do not represent conditions in vivo From a review of methods for such studies (101) it appears that phagocytosis is promoted if allowed to proceed on a base of freshly excised tissue e g lung specimens fixed with formalin or on an inert surface such as that of moistened filter paper the phagocytes can then ingest the particles by trapping them against the walls

Scratched glass (the bottoms of small glass bowls) was used as a base for the phagocytes in the present method According to Myrvik *et al* (77) about 8–16 million cells can be obtained from the lungs with this technique a range that embraces the total of 12–16 million cells obtained in the present study Acording to Myrvik s investigations the great majority of these cells are macrophages less than 0 1 % are polymorphonuclear cells and still fewer are small lymphocytes A predominance of macrophages was also found in the present series

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The choice of exposure technique lies between inhalation via the mouth the nose or a tracheal tube or else the particles can be introduced to the lungs by means of intratracheal injections

If the aerosols are inhaled via the mouth or nose a great deal of the particles will be deposited there Their subsequent transport through the oesophagus may make it impossible to follow the initial phase of lung clearance by external measurement.

Both *tracheal injections and inhalation via* a tracheal tube naturally have the disadvantage of not being physiological Tracheal injections are usually performed by introducing a narrow tube or capillary down into the trachea and then injecting the particles suspended in a fluid This technique has the further disadvantage that the particles are not distributed in the same manner as in normal inhalation Moreover the tube may affect the mucosa, secretion and other physiological *conditions* while the alveolar fluid and permeability may be altered by the suspension. The method has in fact been criticised by several researchers who report that the conditions for retention and deposition differ from those during normal inhalation (5 13 16 17 19 37 57 71)

Inhalation via a tracheal tube gives a better distribution of the particles in the lungs though this is still not identical with the distribution under normal conditions For one thing the animal has to be anaesthetised and this plus the mechanical irritation from the tube alters the respiration (99) moreover turbulence from the vocal cords is reduced and the introduction of a tracheal tube may affect the secretion and possibly *injure the mucosa as well.*

Although the mucoserous glands could not be stimulated experimentally by local irritation of the tracheal mucosa (35) this does not mean that a mechanical irritation cannot have such an effect Nadel & Widdicombe (78) have reported that mechanical irritation of the laryngeal mucosa has the effect of increasing airway resistance Concerning an effect on the secretion however they point out that "the rapid recovery of total lung resistance to control values makes it unlikely that bronchial secretion of mucus was involved"

Several reflexes have been described for the smooth musculature *the blood circulation* and the respiratory dynamics in the respiratory tract (24 78 99) and contraction of the glottis has been reported as one of the results of mechanical irritation of the tracheal epithelium (99)

The tracheal epithelium is highly susceptible to trauma and, even though it has a good healing capacity (50 51) its sensitivity serves to emphasise the disadvantage of introducing a tracheal tube in studies of lung clearance

Tracheal intubation was nevertheless used in the present investigations to facilitate a study of the initial rapid phase of clearance without any disturbance from material deposited in the nose mouth or nasopharynx As already mentioned deposition in these regions would lead to an early increase in the activity in the oesophagus and interfere with the measurement of the initial clearance from the lung

When studying lung clearance fo[illegible] ent sizes of particle simultaneous[illegible] single animal as well as when [illegible] effects with exposed and cont[illegible] consequences of introducing [illegible] should be approximately t[illegible] sizes of particle and both [illegible] respectively

In order to obtain [illegible] practice, the amount [illegible]



Fig 19 Plast c moulds for keeping the rabbits in a fixed position during profile scanning with apparatus nos 1 and 2

tional scaler measurements of retention on isolated lungs The chief disadvantage was the dependence on distance Therefore to improve the technique several modifications were developed

In the second apparatus a focusing slit collimator was introduced to reduce the de pendence on distance while still placing the detector unilaterally This arrangement im proved the isosensitivity characteristics In the light of this experience two focussing collimators and larger crystals were incorpo rated in the apparatus

The validity of the results obtained using the latest profile scanning units is based on experience gained with the earlier apparatus and consequently all four arrangements will be presented below It should be mentioned however that the applied experiments with the apparatus reported in Chapter 7 were conducted with the most developed unit (apparatus no 4)

During the measurements the animal was placed in a special mould (Figs 19 20) which permitted only minor movements of the head This was done to ensure equivalent measuring conditions on different occasions Profile curves that reproduce the distribution of activity over the animal s length were ob tained by coupling a recorder to the detector via a ratemeter The evaluation of these results was facilitated by selecting a rela tively long time constant in relation to the scanning rate This means that the scan curves have a more even course at the expense of the amplitude of the deflection of the re

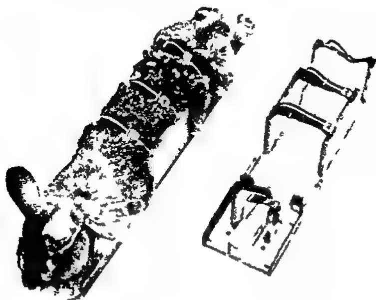


Fig 20 Plastic moulds for keeping the rabbits in a fixed position during profile scanning with apparatus nos 3 and 4

corder In all cases the operating conditions for the detectors photomultiplier were adjusted to the activities in question The apparatus was calibrated daily by performing measurements on known standard specimens

Apparatus no 1

The first profile scanning unit (Fig 21) was fitted with a detector (Tracerlab P 20QG) with an NaI crystal (1″ × 1″) placed under the animal and a slit shaped lead collimator (LKB 337035) with the slit confined by a superimposed rectangular lead shield with an aperture of 2 × 50 mm The detector was connected via a ratemeter (Tracerlab SC 79 205) to a recorder (Varian G 11 A/A_2) into which the paper was fed at a constant rate A relatively long time constant (40 sec) was selected for the ratemeter in order to minimise the statistical variation in the count rate

The detector, vertically orientated was mounted on a carriage fitted to a longitudinal screw in the stand This screw driven by a synchronous motor caused the detector to travel at a constant rate of 0 5 cm/min along the under side of the animal After scanning the detector could be returned to its initial position to start a new scanning in a cranio caudal direction The field of measurement i e the animal s thorax region lay 2 5–9 0 cm above the collimator

Apparatus no 2

Apparatus no 1 was improved by using a specially designed slit "focusing" collimator (Fig 22) In this case the scintillation detector (Tracerlab P 20 QG) equipped

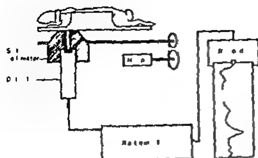


Fig. 21 Diagram of profile scanning apparatus no. 1 (see text)

with an NaI crystal (1″ × 1″) was coupled to a writer (Varian G 11 A) via a ratemeter (Tracerlab SC 34 A) with a time constant of 40 sec. Chart speed was 0.6 cm/min.

The detector was mounted in the same way as in the first unit using a carriage fitted to a longitudinal screw and driven at a constant rate along the animal by a synchronous motor. After scanning the detector could be returned to its initial position either automatically or manually. Scanning could be performed at a single constant rate (2.5 cm/min). The field to be measured lay 2.5–9.0 cm above the collimator.

Apparatus no. 3

This profile scanning unit (Figs. 23, 24) was fitted with two opposed focusing slit lead collimators in the same plane each with a scintillation detector (Harshaw integral line assembly type 1258/*a*) having a 3″×2″ NaI crystal. The movements of the collimators and recordings were sychronised. The count rate was read via a two channel gamma spectrometer (Swedish Atomic Energy Co. type 3203), with each channel coupled to a writer (Varian G 11 A). The ratemeters operated with a time constant of 40 sec. The photo peak for Au^{198} was read at 0.41 keV and for Sc^{46} at 0.89 keV with a channel width of 20 keV in both cases. The photomultipliers of the detectors operated under matched conditions adjusted to the activities in question.

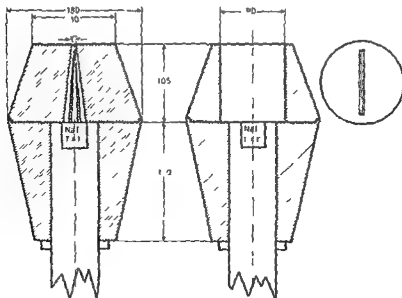


Fig. 22 Focusing slit collimator used in profile scanning apparatus no. 2

Fig. 23 Diagram of profile scanning apparatus no. 3 (see text)

Fig. 24 Photograph of profile scanning apparatus no. 4 (see text)

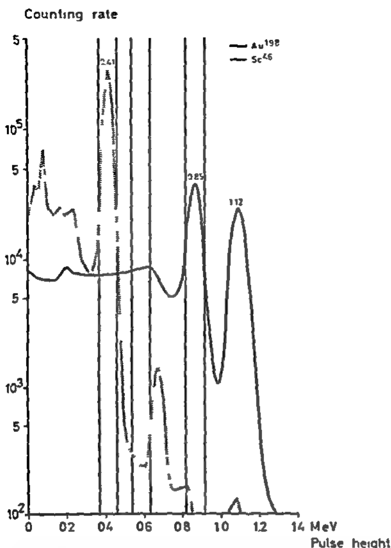


Fig 25 The γ spectrum of Au^{198} and Sc^{46} measured with a 3" × 3" NaI crystal The position and boundaries of the three channels used for reading the γ-energies of the two isotopes simultaneously are given with vertical lines The channel at 0.89 MeV ± 0.05 is used for reading the counts of Sc^{46} from its photopeak The channel at 0.58 MeV takes up as many counts from Sc^{46} as is represented in the channel at position 0.41 MeV—the photopeak of Au^{198} This is arranged by justifying the width of the channel at 0.58 MeV in such a way that the area representing the counts of Sc^{46} in this channel will be as large as that in the channel at 0.41 MeV (these areas are shaded in the figure) The counts of the photopeak of Au^{198} in the channel at 0.41 MeV ± 0.05 are then read as the difference between the total counts and those coming from the Sc^{46} in this channel In apparatus no 4 this was arranged automatically the counts in the 0.58 MeV channel being subtracted from the total counts in the 0.41 MeV channel The number of counts from Au^{198} that occur in the 0.58 channel is negligible

HIGH-VOLTAGE POWER SUPPLY
PRE-AMPLIFIER
2-CHANNEL ANALYSER
SCALER
TIMER
SCALER
TIMER
3 x 3" NaI CRYSTAL
CONICAL COLLIMATOR
IRON PLATES

Fig 26 Diagram of the whole body counter

Apparatus no 4

A further improvement was achieved by having a third channel in the measuring unit of apparatus no 3 This third channel was coupled to give an automatic reduction of the interference from the energy spectrum of Sc^{46} at the level at which the photo peak for Au^{198} was read This was done by setting the third channel with a suitable width to a level between 0 41 keV (the photopeak of Au^{198}) and 0 65 keV (the limit for the first relatively steep decline in the gamma energy spectrum of Sc^{46}) The activity measured in the third channel could thereby be adjusted so as to be equivalent to the interference from Sc^{46} in the channel for Au^{198} (Fig 25) A channel width of 100 keV was used for the channels at photopeaks of 0 41 and 0 89 keV respectively

Whole body counter

The long term studies of lung clearance were

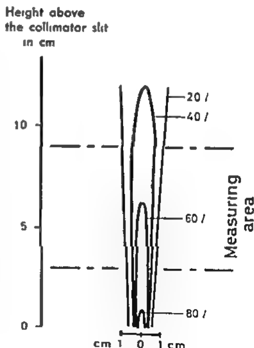


Fig 27 Au^{198} isoresponse curves in air obtained with the slit collimator of profile scanning apparatus no 1

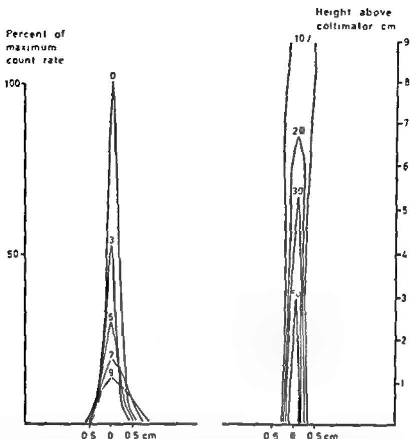


Fig 28 Au^{198} scan and isoresponse curves in air obtained with the focusing slit collimator of profile scanning apparatus no 2 The figures in the scan curves refer to the distance between the collimator and the point source The figures in the isoresponse curves refer to equal count rates

performed with an open type of whole body counter designed and constructed at this Institute (Fig 26) and consisting of a 3″ × 3″ NaI crystal surrounded by a lead collimator with a conical aperture pointing downwards The collimator was mounted on a stand at a height of 60 cm above a sheet of iron 15 cm thick The collimator and iron sheet effectively screened the direct background radiation, which was thus only 0 07 cps in the Sc^{46} channel The detector was powered by a high tension unit (Oltronix LS 524) and was coupled to a scaler (Philips PW 4032) and timer (Philips PW 4052)

Characteristics of the apparatus

In order to make an adequate assessment of the measurements obtained it is necessary to know about the various characteristics of the apparatus The characteristics of the various profile scanning units are considered below on the basis of investigations conducted on radioactive specimens in air and water as well as on specimens in frozen rabbit cadavers

Apparatus no 1 was used to test the applicability of the profile scanning technique in lung clearance studies The isosensitivity of the collimator in air was studied

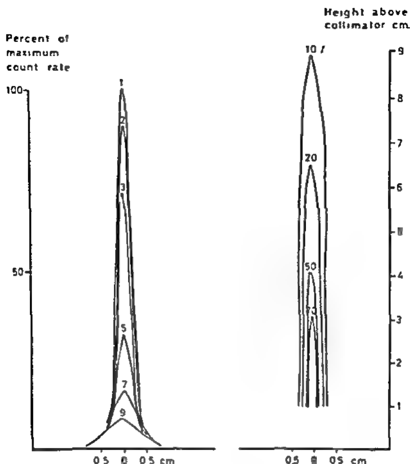


Fig 29 Au^{198} scan and isoresponse curves in water obtained with the focusing slit collimator of profile scanning apparatus no 2 The figures in the scan curves refer to the distance between the collimator and the point source The figures in the isoresponse curves refer to equal count rates

The characteristics of apparatus no 2 were investigated in greater detail The isosensitivities in air and water were determined for apparatuses nos 3 and 4

Collimator, Apparatus no 1

The isoresponse curves are shown in Fig 27 with respect to the isosensitivity in air for Au^{198} The curves represent count rate levels for a point shaped Au^{198} specimen in a vertical plane centered on and at right angles to the collimator aperture The isoresponse curves were plotted from measurements of the count rate from a point shaped Au^{198} specimen placed in a total of about 60 different positions in the measuring plane

Collimator Apparatus no 2

Selectivity

The collimator s selectivity was determined with stepwise Au^{198} scan curves and isoresponse curves in air and water (Figs 28 29) In the latter case this was done to simulate the conditions for external measurements

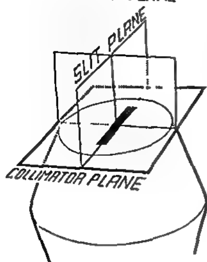


Fig 30 Diagram to show the different planes referred to in the study of the response characteristics of the collimator in profile scanning apparatus no 2

on rabbits since the biological tissues change the energy spectrum of the radiation that reaches the detector (85)

For these studies, point shaped specimens (ca 2 μc) were placed in selected positions in a measuring plane at right angles to the collimator aperture and the collimator plane (Fig 30) The stationary scan curves thus describe the count rate obtained when the specimens are in different positions in the measuring plane The isoresponse curves indicate equal count rates in this measuring plane with the highest count rate (set at 100 %) directly in front of the collimator aperture and at 8 cm above this The isoresponse curves in water were obtained by placing a plastic container (PVC) approximately 2 mm thick with its base 1 cm above the collimator plane and carrying out the measurements as above The highest count rate set at 100 % is represented in this case by a point immediately in front of the collimator aperture and 1 cm above this

Variation with distance

The variation with distance when recording the activity from a point source of Au^{198} was established by means of dynamic measurements (recording of scan curves) with the specimen placed at five selected heights over the centre of the aperture The results are shown in Fig 31 The maximum recorded difference amounts to ± 27 % when the activity from a point source is moved a maximum distance towards or away from the collimator from the mean height of the measuring area Repeated measurements with the same position for the specimen gave variations in the measurements that correspond to a standard deviation of ± 2 6 % This is shown in Fig 31 by the shaded area This standard deviation may be regarded as the error of method for the measuring procedure

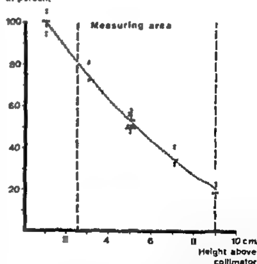


Fig 31 Detector system response shown as a function of the distance between a point source (Au^{198}) and the focusing slit collimator of profile scanning apparatus no 2 The shaded area shows the standard deviation ± 2 6 % of repeated measurements of the area of scan curves obtained from the point source

Power of resolution

The power of resolution was defined as the distance between two specimens that results in a profile curve with two maxima such that the smaller maximum exceeds the minimum between the two peaks by at least three times the range of the background variation in at least 4 out of 5 consecutive measurements

The collimator s power of resolution was determined using Au^{198} specimens in 1 mm plastic tubes all with the same activities but paired in different lengths namely 1 12 5 25 and 50 mm These were placed in line with each other at selected heights in the measuring plane parallel to the collimator plane The distance between the specimens was varied and five continuous as well as five stepwise scannings were performed for each distance

The nearer the two specimens approached one another the more the profile curves overlapped until it became impossible to distinguish two separate fractions In the continuous measurements this occurred when the distance between the point shaped specimens was 11 and 18 mm at 2 5 and 8 5 cm respectively over the collimator In the stepwise measurements the corresponding figures were 2 and 12 mm at 2 5 and 8 5 cm respectively over the collimator

The limiting values for the resolution of the point shaped and the other specimens are given in Fig 32 for the continuous measurements and in Fig 33 for the measurements performed by stepwise scanning

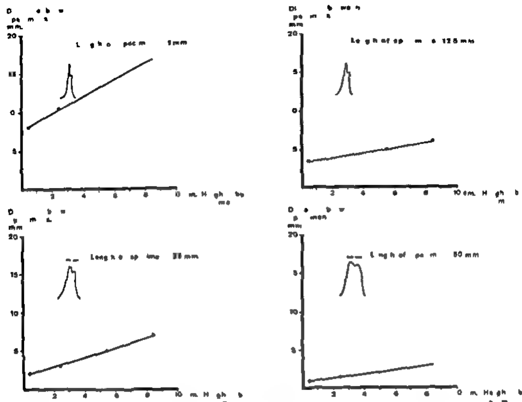
The curves obtained by continuous scanning were evaluated planimetrically as described in Chapter 6 (page 62) using a template designed for these scan curves The evaluation of the profile curve of the point source scanned first gave the same spread as did measurements on equivalent isolated specimens when the distances between the point source specimens were greater than the values given in Fig 32 This means that if the occurrence of two separate profile curves i e approximately equivalent concentrations of radioactivity, is indicated by a curve having two maxima conforming with the above conditions the area of each fraction can be measured with the same degree of accuracy as for measurements of independent curves This is done by covering the area of the fraction scanned first with the template and obtaining the area of the second fraction by subtracting the area of the first from the total area of the profile curve Owing to the time factor in the ratemeter the second fraction is overlapped more by the first than vice versa and consequently it can not be screened off with a template as can *the first fraction*

Since the relevant experiments showed that the distance between the scan fractions for the larynx lungs and stomach amounts to several centimeters it should be possible to distinguish between two nearby fractions from scan curves obtained from Sc^{46} with an adequate margin in experimental studies on animals

Collimator, apparatus nos 3 and 4

The Sc^{46} isoresponse curves in air and water of this collimator arrangement are shown in Figs 34 and 35 The study in water was performed to simulate conditions in biological tissue A comparison of Figs 34 and 35 with Figs 28 and 29 shows that the new arrangement gives an approximately equivalent collimation

The coefficient of variation for measurements of point source standards (± 5 %) of Au^{198} and Sc^{46} is shown in Fig 36 Readings of 50 nc Au^{198} have e g an error of approximately 15 %



F g 32 Limiting values for the d stance resolut on of pa rs of Au^{195} sources of d fferent lengths by cont nuous scanning w th apparatus no 2 (see text)

W hole body counter

The only factor investigated for the whole body counter was its isosensitivity to Sc^{46} in air The curves were evaluated from measure ments of the count rate from a point source of Sc^{46} placed in more than 100 different positions in the measuring planes investi gated The isoresponse curves are shown in Figs 37 and 38 for two mutually perpendi cular planes through and parallel to the axis of the coll mator

Validity of the measurement method for lung clearance studies

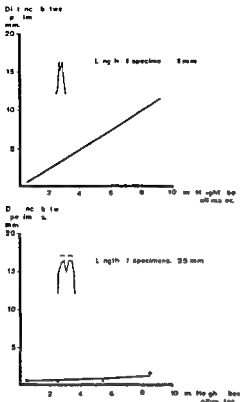
To test the u e of profile scanning units for evaluating the retention of test aerosols in the lungs at d fferent times stud es were per formed correlating this technique with activ ity measurements on isolated lungs A num ber of studies were also carried out on rabbit phantoms to investigate the power of resolu tion of the instrument with respect to ac tivit es in nearby organs etc

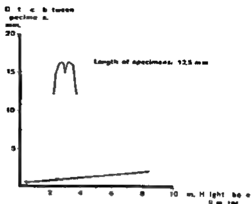
Profile scanning apparatus no 1

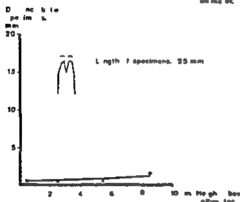
Correlation between profile scanning and radioactivity measurements on isolated lungs

Method

Stud es were performed us ng the 28 rabb ts for the invest gation of the tagg ng isotope s translocat on from gelat n part cles (Chapter 3) Dur ng the exposure wh ch lasted for about 30 min the rabb ts were kept in plast c bags in order to prevent their fur from becoming contam nated After the exposure the plastic bags were removed and the







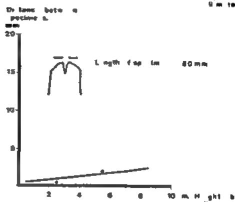


Fig 33 Limiting values for the distance resolution of pairs of Au^{198} sources of different lengths by continuous scanning with apparatus no 2 (see text)

animals were placed in special plastic moulds (Fig 19) designed to fit their body in the resting position The animals were immobilised by these moulds being only able to move their heads vertically a couple of centimeters At different intervals after exposure (¼ ½ 1 2 4 8 and 16 hours) the animals were sacrificed in groups of 4 immediately after three profile scannings had been performed with apparatus no 1 the ratemeter being set at a time constant of 10 sec The animals were killed with pentobarbital given intravenously the lungs and other tissues were removed at once wet ashed in concentrated HNO_3 and evaporated to 10 ml

The profile curves were evaluated by planimetry as described in Chapter 6

Since the profile scanning was performed immediately before the animals were sacrificed a direct comparison could be made between the two methods for measuring the activity in the lungs

Results

Fig 39 shows the correlation between measurements of the activity in the lungs made directly from isolated lungs and by profile counting over the lungs in the living animal The coefficient of correlation was 0 97

Profile scanning apparatus no 2

Evaluation of different distributions of radioactivity studied in rabbit phantoms

Lung surface and bronchial activities

In order to determine the maximum variation between the scan curve areas for activ

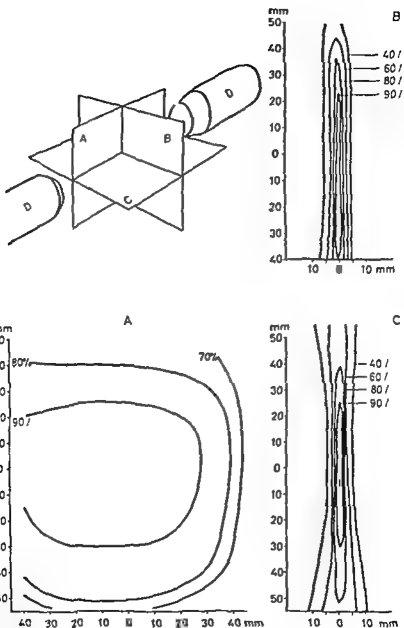


Fig 34 Au^{198} isoresponse curves in air for the collimators in profile scanning apparatus nos 3 and 4 The different planes studied are shown up to the left.

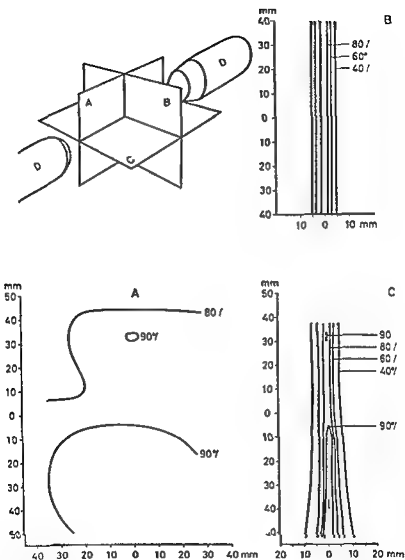


Fig 35 Au^{195} isoresponse curves in water for the collimators in profile scanning apparatus nos 3 and 4 The different planes studied are shown up to the left.

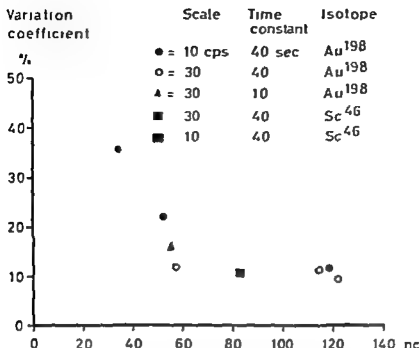


Fig 36 The coefficient of variation for the area of scan curves obtained from an absolute calibrated (± 5 %) standard point source of Au^{198} and Sc^{46} (from profile scanning apparatus no 4)

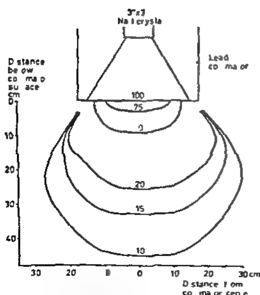


Fig 37 Sc^{46} isoresponse curves in air for the collimator in the whole body counter

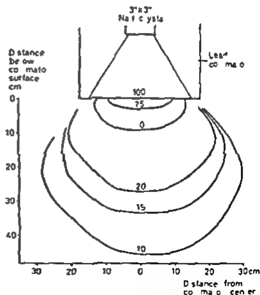


Fig 38 Sc^{46} isoresponse curves in air for the collimator in Fig 37 in a perpendicular plane

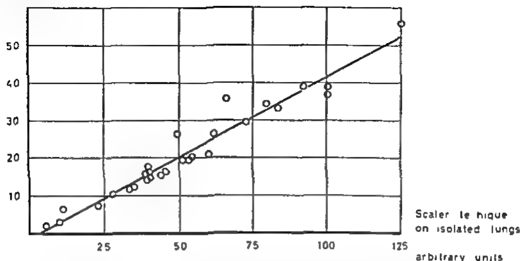


Fig 39 S atter diagram and regression line demonstrating the correlation between radioactivity measured by scaler technique on isolated lungs and planimetrically evaluated areas of lung scan curves obtained with profile scanning apparatus no 1

ities deposited in extreme positions in the lung experiments were performed using 4 rabbit lungs A comparison was performed between activities on the surface and in the bronchi of lung phantoms

After the lungs had been dissected free inflated to normal size and dried two of them were cut up into horizontal sections approximately ½ cm wide Equal amounts of activity (approximately 4 μc) were then applied bronchially (from 1 cm over the carina down to and including the fourth bronchial division) in as similar a manner as possible to both specimens using a micrometer syringe The other two lung specimens had the same amount of activity evenly applied to their surfaces Rabbit phantoms were produced by sacrificing rabbits with pentobarbital placing them in measuring moulds and then refrigerating them at –40°C.

The frozen animals were sawn lengthwise into two halves after which the lungs were chiselled out and replaced with the specimens described above these had been painted with a thin layer of plastics to protect them from moisture

Profile scanning of these phantoms showed that the scan curve area from the bronchial activity was 17 % larger than the corresponding value for the "peripheral" activity i e that applied to the surface of the lung specimens

Activity in lungs and stomach

Lungs prepared in the same way as above were used in an attempt to determine the influence of known amounts of activity located in different parts of the stomach upon readings of a known activity in the lung

In these experiments 5 sacrificed rabbits were frozen to –40°C and then sawn lengthwise into two halves after which the lungs were chiselled out. Lung phantoms with bronchial activity (approximately 5 μc) and impregnated with plastics were placed in three of these rabbits The other two received lung specimens with the activity (approximately 4 μc) distributed over the sur face Point sources of activity—46 and 91 % re

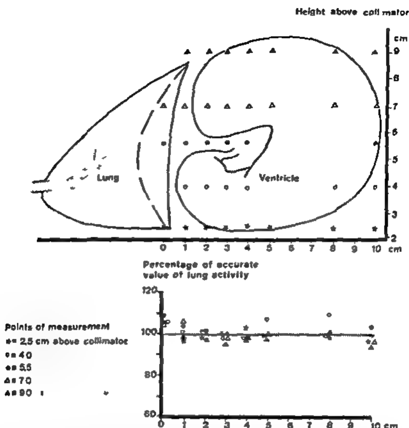


Fig 40 The diagram in the lower part of the figure illustrates the effect of a point source of Au^{198} in different positions in the stomach region on the simultaneously scanned activity in a lung phantom with bronchial application of Au^{198}. The different positions of the point source are indicated in the upper part of the figure. The activity of the point source was approximately 46 % of the activity in the lungs

spectively of the lung activity in the "bronchial" and "peripheral" lung phantoms—were placed in different positions in and outside the stomach and the effect of this on the recordings of lung activity was studied

The interference from activity evenly distributed in the stomach was studied in the same way except that the stomach chiselled out from the rabbit phantom was replaced by an oblong rubber bladder filled with a gold chloride solution tagged with Au^{198} amounting to 77 and 91 % of the activity in the bronchial and peripheral lung phantoms respectively

The results are illustrated in Figs 40 and 41 *which show that a point shaped activity* —situated ventrally against the diaphragm—of the same size as the activity in the "peripheral" lung specimen gives an increment of approximately 14 % to the reading of the lung activity In reality however this site is occupied by the liver The increment 1 cm caudally of this position was negligible In the case of bronchial activity in the lung specimens an increment of 10 % was ob

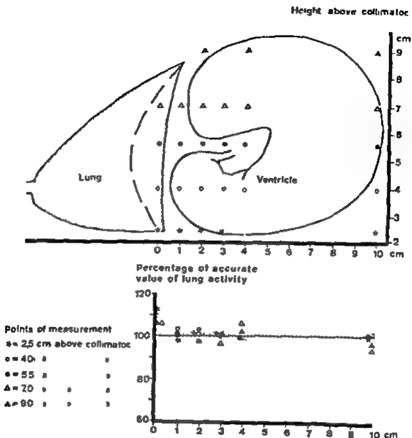


Fig 41 The same diagram as in Fig 40 with Au^{198} applied to the surface of the lung phantom The activity of the point source was approximately 91 % of the activity in the lungs

tained when the point source lay against the diaphragm

The readings of activity in the lungs were not affected by activity evenly distributed in the stomach phantoms and amounting to 77—91 % of the lung activity

Activity in lungs and trachea A similar study was made of the interference from activity in a trachea phantom on a lung phantom in which the activity (approximately 4 μc) was distributed "bronchially" The trachea phantom's activity was evenly distributed in this and amounted to 42 % of activity in the lung specimen In this case area of the lung scan curve increased only 7 %

Profile scanning apparatus nos 3 and

No studies were made concerning the val ity of measurements performed with th apparatuses Nor do such studies appear be necessary considering that these ur were superior to apparatus no 2 (which v investigated) with respect to factors such

dependence on distance and isoresponse of the detector system

Discussion

The analyses of isolated lungs confirm the assumption that the "lung peak" observed in profile counting was in fact due to the radioactivity in the lung

The correlation with the scaler technique showed that profile scanning is an appropriate method for lung clearance studies on experimental animals provided that the selectivity of the collimator reduces the interference from activity in adjacent organs to an acceptable level

The primary advantages of external methods for measuring lung clearance in vivo compared with conventional techniques on sacrificed animals is that clearance can be followed and repeated studies be performed on one and the same animal and that consequently one requires a smaller number of animals Another advantage is that transport of particles from one organ to another can be studied with a single detector

Profile scanning is not dependent on exact equivalent geometry concerning the animal s location in relation to the detector system as is the case when using a stationary detector system In the latter case the measuring field often cannot include the entire lung owing to the risk of including parts of the stomach as well

A disadvantage of profile scanning is that one cannot study such small amounts of radioactivity as with a fixed detector The method is therefore chiefly confined to the study of clearance immediately after exposure and during the first week or so after this

Another disadvantage common to all methods for external measurement is that one cannot determine the exact location of the amounts retained As a rule moreover no direct measure is obtained of the initial deposition which consequently has to be calculated by extrapolation of the values for retention at different intervals after exposure

The variation with distance is of course a disadvantage in unilateral recording of the radioactivity from a source in an organ particularly if the source comes extremely close to or far away from the detector The further away the detector moves from the organ, the smaller becomes the difference between the recordings of activity from the nearest and furthest parts of the measurement area at the same time as the efficiency and power of resolution for the measurements deteriorate

In the present study with apparatus no 2 the variation for the measurement area in question amounted to ± 27 % in recordings of the activity from a point source moved to the extreme dorsal or ventral positions from the centre of the measurement area Owing to the symmetry of the lungs this disadvantage can be offset to some extent by placing the detector against the animal s side or by using two or more detectors placed symmetrically around the animal Still better conditions are obtained by using focusing collimators, which are less dependent upon distance as was done in apparatuses nos 3 and 4

The experiments with apparatus no 2 and frozen rabbit phantoms show that the difference between equal amounts of activity applied "peripherally" and "bronchially" respectively to dried lung specimens amounted to only 17 % for such extreme distributions The investigations presented in Chapter 3 show that the redistribution of the retention of 3 and 6 μm particles between the initial phase and 72 hours after exposure is nothing like as great as the conditions represented by these phantom studies Consequently the difference in recordings of activity from the

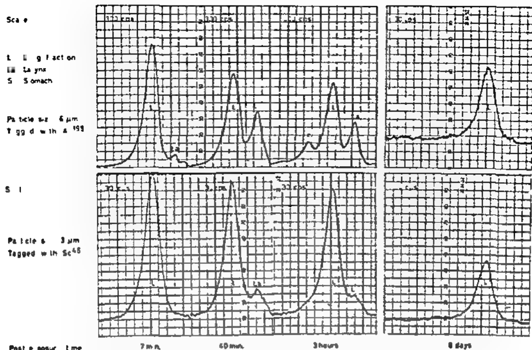


Fig 42 Profile scan curves from rabbits exposed to 3 and 6 μm polystyrene tagged with Sc^{46} and Au^{198} respectively The radioactivity is gradually transferred from the lung area (L) to the larynx (La) and then to the stomach (S) area later on it disappears This transportation process is faster for the bigger particles In this animal the remaining radioactivity of the two isotopes was confined to the lung area after 8 days from then on lung clearan e can be accurately measured by whole body counting

aerosol initially retained in the lung after inhalation via a tracheal tube compared with possible distributions in the lung at different times after this will not give rise to such large discrepancies in practical experiments as those noted here

The collimator used in apparatus no 2 and the technique employed for distinguishing between the different fractions of the profile curves have made it possible to record the activity in the lungs without any essential interference from activities in adjacent organs such as the larynx trachea and stomach In the phantom studies when the activity in the trachea amounted to 42 % of that in the lung specimen the increment to the lung scan curve was only 7 % Although no direct conclusions can be drawn from the investigations reported and discussed in Chapter 3 the data do suggest that the disturbance from activities in the trachea during an initial phase of clearance can approach the same magnitude as noted here provided that the activity is evenly distributed in the trachea.

The phantom studies also showed that even with an extremely unfavourable location of the activity in the stomach—a point source against the ventral side of the diaphragm amounting to 91 % of the activity in the lungs—the readings of "bronchially" distributed activity in the lungs were affected by less than 10 %

The maximum effect of interference from the transport of activity in the oesophagus on readings of lung activity will correspond to the amount that passes through the oesophagus during measurement In the investigations reported in Chapter 3 only negligible activities were noted in the oesophagus Moreover since profile scanning makes it possible to detect any larger quantities that pass through the oesophagus the interference from these can be avoided

With the aerosol in question the crystal (NaI 1" × 1") and the collimator in apparatus no 2 studies were limited to initial retentions in the milligramme range (53) Apparatuses nos 3 and 4 permitted studies of clearance with initial retentions down to 0 2 μg At the expense of poorer resolution, a wider aperture could be used to increase *the efficiency of the measurements* How ever the planimetric evaluation of the profile curves (cf Chapter 6) would be more difficult and consequently a relatively high power of resolution was chosen even though this meant using a relatively slow scanning rate

In order to follow lung clearance in live animals over periods running into months one needs to be able to measure small amounts of radioactivity This can be done with a whole body counter as a complement to profile scanning Whole body measurements cannot be used for this purpose as long as there is interference from radioactivity in other organs It can, however be employed once profile scanning has shown that the lungs contain the greater part of the radioactivity in the animal Fig 42 illustrates how this time can be determined The figure reproduces profile scan curves from a rabbit exposed simultaneously to 6 and 3 μm polystyrene particles tagged with Au^{198} and Sc^{46} respectively, and demonstrates how the radioactivity moves from the lungs to the laryngeal region and from there to the *gastrointestinal tract Later day 8 in this case* radioactivity is found only in the lungs Beyond this time period whole body measurements will accurately reflect the lung clearance

CHAPTER 6

EVALUATION OF THE MEASUREMENTS

Profile scan curves

The curves recorded with the profile scanning technique represent the distribution of the radioactive tagging substance along the length of the animal studied The appearance of the curves varies with the time that has elapsed since exposure to the test aerosols As a rule several different fractions can be distinguished corresponding to concentrations of the tagging substance in different parts of the animal These fractions generally overlap to some extent Fig 42 provides an example of the appearance of scan curves for an animal 7 and 40 minutes 3 hours and 8 days after the end of exposure to a di disperse (6 and 3 μm) polystyrene aerosol The figure illustrates how the lung fraction decreases with time as the activity is displaced towards the larynx and later down into the stomach and intestines

For a particular distribution of the activity in the direction of scanning both the surface under the curve and its height are proportional to the activity scanned This can be verified mathematically for instance for activity concentrated to a point Both relationships have been utilised for evaluating the lung activity and their validity has been tested in a series of experiments in which the lungs of exposed rabbits were measured by profile scanning immediately before being removed wet ashed in HNO_3 and measured for their total radioactivity using a conventional technique

Overlapping by nearby fractions complicates the evaluation of the scan curves particularly if a time lag is involved in the form of a time constant to minimise the statistical variation in the count rate The time constant causes a lag in the scan curves in relation to the location of the activity measured The effect of this however is the same for all scan curves that do not overlap each other A scan curve that is overlapped by another curve positioned before it in time will however, get a reduced lag The degree of this reduction depends on the distances between the different sources and the relations between their activities as well as on the isoreponse characteristics of the collimator system used Allowance must be made for these circumstances if the locations of the activities are to be determined exactly This was not the aim in the present investigation The identification of the main fractions e g larynx lung and stomach is not however affected The distance between e g the larynx peak and the lung peak on the scan curves is approximately three times the time constant It has been found that these factors do not reduce the time lag in the lung peak to any measurable extent for the relation of activities found in the studies performed

The overlapping considerably complicates the mathematical relationship mentioned above making it difficult to perform a mathematical analysis of the conditions involved The surface distribution of the different fractions must therefore be determined

empirically on the basis of principles derived from experiments

Surface measurement with a planimeter

The area of the lung scan fractions was calculated by reconstructing the appearance of the isolated fractions and measuring their area with a planimeter

Templates were specially constructed for evaluating these areas For each isotope studied a template was designed on the basis of a number of independent scan curves obtained from exposed rabbits in which the aerosol was deposited only in the lungs These scan curves thus reproduced the true appearance of the lung fraction i e without interference from radioactivity in other organs Profile curves from lungs with different retentions were compared (Fig 43) and it was found that if a curve was shifted sideways its shape between the point of inflection and the base line was very similar to this distance on a higher curve This circumstance made it possible to construct templates with which the curve of the lung fraction could be isolated from curves of overlapping fractions

The validity of the planimetric calculation of lung scan curves obtained in this way has been presented above (cf p 51) The experiments demonstrated that the two methods gave similar results the correlation coefficient being 0 97 Fig 44 *A* illustrates how the lung fraction was separated from adjacent fractions on a profile scan curve obtained in the experiments on animals

The study on rabbit phantoms (cf p 55) using a point source of radioactivity placed in various positions in the gastric region showed that this did not interfere with the readings of radioactivity in the lung when the latter was distributed bronchially or on the surface

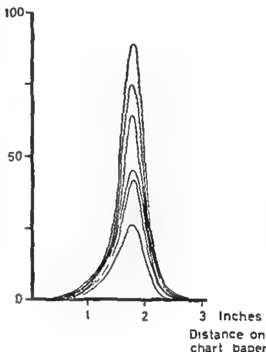


Fig 43 Scan curves from animals with different retentions of 3 μm Sc^{46} tagged polystyrene particles showing the almost identical slope between the base line and inflection point on one curve and the same distance on the lower part of the next larger curve

Height measurement of lung fraction

Since the planimetric calculations are time consuming an attempt was made to develop *a more simple method for evaluating the profile curves* For this purpose curves obtained from animals measured in the same way were classified into the following five main types

1 A pure lung fraction
2 A lung and a "larynx" fraction
3 A lung and a stomach fraction
4 A "larynx" a lung and a stomach fraction

Scale

L Lung fraction
La Larynx
S Stomach

Particle size 6 μm
Tagged with Au 98

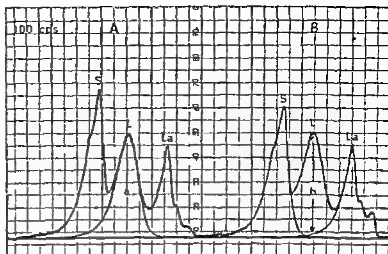


Fig. 44. A: the lung fraction area (*a*) on an original scan curve screened off from the total scan curve using a template constructed on the basis of the curves in Fig. 43. The template is used to draw the line from the point of inflection down to the base line on the profile curve as shown in the figure. *B*: the height (*h*) of the lung fraction measured after allowing for the influence of radioactivity in the larynx and stomach. Both scan curves were obtained from the same animal with an interval of approximately 15 minutes.

5. Complex curves (indistinguishable fractions).

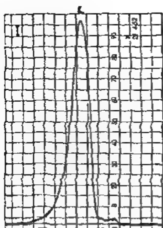
Examples of the various types of profile curves are shown in Fig. 45.

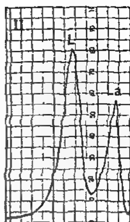
A computerised study was made of the correlation between the area *a* of the lung fractions as calculated planimetrically and the peak height *h* of the fraction above the background level.

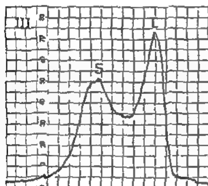
In the case of type 1 curves, in which there was no interference from adjacent fractions, a direct comparison could be made between *h* and *a*.

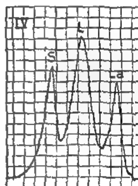
For types 2 and 4, interference from the laryngeal and gastric regions respectively had to be eliminated before the height of the lung fraction could be calculated. A number of "larynx" scan curves were studied in order to establish the size of this interference. The method previously employed for constructing templates with which to isolate the lung scan fractions could only be used in the very few cases in which the laryngeal fraction was relatively isolated. Instead, therefore, the most probable appearance of the caudal side of the laryngeal fraction was estimated from the discharge function of the ratemeter condenser at the time constant used. The shape of the curve obtained in this way was then used in the same manner as for the lung scan fractions to reconstruct the appearance of the fraction from its caudal point of inflection to the background level. The height of the lung fraction could now be measured as the vertical distance between its maximum and its intercept with the curve of the laryngeal fraction (Fig. 44 *B*).

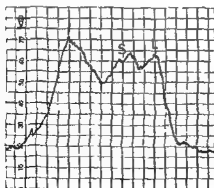
Type 3 curves, besides being treated in the same way as types 2 and 4, were also analysed for the probable appearance of the oral side of the stomach fraction, which was plotted in a similar manner. In most cases the stomach scan curve did not overlap as far as











I–V = Different types of scan-curves

L = Lung fraction
La = Larynx
S = Stomach
I = Interstine

Fig 45 Main types of profile scan curves obtained from different animals (see text)

TABLE 4 Correlation between height measurement and planimetric evaluation of scan curves

Type of scan curve	Number of observations		Coefficient of correlation		Standard deviation of b given a (mm)		h axis intercept in mm	
	Au^{198}	Sc^{46}	Au^{198}	Sc^{46}	Au^{198}	Sc^{46}	Au^{198}	Sc^{46}
1	18	57	0 98	0 99	1 7	2 9	2 4	2 1
2	199	198	0 99	0 99	3 9	4 2	2 3	1 6
3	173	220	0 99	0 99	3 3	3 1	2 5	0 4
4	317	268	0 98	0 99	3 4	3 4	1 5	1 5
5	38		0 92		7 1		0 0	

to the perpendicular through the peak of the lung scan curve

Type 5 curves were analysed in the same way as type 4 The results were somewhat unreliable, however because very approximate estimations had to be made

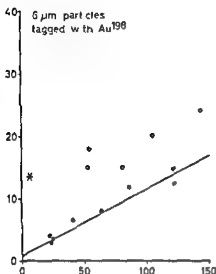
The correlation study showed that the heights were proportional to the surface areas of the lung fraction The correlation coefficients between b and a for the different types of scan curves are given in Table 4 together with the standard deviation for b at a given a and the intercept with the b axis

The height of the scan curves in the experiments varied between 15 and 125 mm Table 4 shows that the intercepts on the b axis are small compared with the standard deviations indicating that the use of height instead of planimetric data does not introduce any considerable systematic error Such errors can be avoided entirely by using a regression line to convert the height data into areas corresponding to those obtained by planimetry

The studies of deposition and translocation reported in Chapter 3 provided a direct comparison between the height of the lung scan fractions measured in the above manner and the activity in isolated lungs The correlation of these data is shown in Fig 46 It will be seen that the Au^{198} levels for rabbits 01—05 (which were sacrified soon after exposure to 3 and 6 μm monodisperse polystyrene particles tagged with Sc^{46} and Au^{198} respectively) are clearly separated from the other levels as is the data for rabbit 21 sacrificed two hours after exposure A deviation was expected in the case of rabbits 01–05 because it took about 30 minutes to sacrifice the animals and remove their lungs without allowing them to collapse Since these particles have a high initial rate of clearance a certain amount of radioactivity may have disappeared from the lungs during this time Data on the larger particles in these rabbits were therefore excluded from the correlation calculations In the case of the smaller particles tagged with Sc^{46} the levels at zero time were included in the correlation calculations because as shown by the retention study they have a lower deposition that the larger particles at the tracheo-bronchial level

The activity from Au^{198} in the trachea of rabbit 21 amounted to 33 4 % of that in the lung at the same time (Table 2) On the scan curve recorded just before the animal was sacrified however the height of the lung fraction was approximately twice that of the tracheal fraction indicating that a large proportion of the Au^{198} activity was transferred to the trachea when the lung was

Profile scan
of lung
Height in
arbitrary units



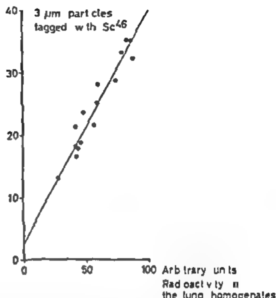


*Fig 46 Scatter diagrams and regression lines demonstrating the correlation between the radioactivity of Au^{198} and Sc^{46} (in 6 and 3 μm particles respectively) as measured by scaler technique on isolated lungs (homogenised in HNO_3 and H_2SO_4) and as evaluated from the height of the lung scan curves obtained with profile scanning apparatus no 4 * rabbit no 21 o the group of animals killed at zero time after exposure The discrepancies are explained in the text*

manipulated during dissection This transfer may have taken place for instance with the bronchial secretion which contained most of the particles tagged with Au^{198} Under these circumstances the data from this rabbit were excluded when calculating the correlation reported in Fig 46

Discussion

Essentially three techniques have been used for evaluating the different fractions of scan curves The fractions have been divided by perpendiculars through the minimum levels between the peaks or their true appearance has been reconstructed i e their appearance after eliminating the effect of adjacent fractions In other cases particularly when recordings have been made with a short time constant the peak height of the fraction above the base line has been used The last two methods have proved applicable in the present studies The first one was not suitable because the time constant used for the ratemeter produced a lag in the curves resulting in a skew distribution Consequently, a perpendicular division of the fractions meant that the part cut away from the fraction in question was not fully compensated for by the inclusion of part of the adjacent fraction

The area under the scan curve and the height of the curve are proportional to the activity scanned In a mathematical analysis

this means that evaluations based on the area or height of the lung fraction curve are not completely correct unless any changes in the distribution of activity in the lung occur uniformly along the longitudinal axis

Such deviations from this characteristic distribution of activity certainly do exist between the distribution of aerosols retained in the lungs of different individuals and at different times in a single individual (cf pp 24—31) As the present correlation studies show however this distortion appears to be negligible with the measuring technique used

Measuring the height of the lung fraction is a simpler procedure than the planimetric evaluation of its area Both methods are affected by changes in the position of the animal attempted kicking etc or variations in the detectors rate of travel Scan curves subject to errors of this sort have not been included in the analysis Moreover a few scan curves incorporating other disturbances of unknown origin (resulting in type 5 curves) were also excluded from the lung clearance studies reported in Chapter 7 Curves excluded for these reasons comprised about 2—3 % of all those obtained

Variations in the count rate of background activity were so small that they did not interfere with the evaluation of the area or peak height of the scan curves This is clear from the correlation studies in which any interference from such variations is included in the recorded data

Whole body measurement

Whole body measurement was started when profile scanning showed that no appreciable amounts of activity remained in the gastrointestinal tract As a rule this occurred 5—10 days after the exposure to test aerosols It was not possible to follow the long term clearance of the 6 μm particles tagged with Au^{198} owing to the short half life of this isotope Studies with the whole body counter were thus limited to the clearance of 3 μm particles tagged with Sc^{46} during rather more than one month after exposure

During these measurements the animals were placed in special moulds which prevented them from changing position The measuring times which were calculated so that the statistical error in the observations was less than 2 % amounted to 10—30 minutes The results were corrected for the physical decay of the isotope and converted into the same type of arbitrary units as for the profile scanning data

Mathematical models

In most studies concerning the lung clearance of solid particles that are relatively insoluble in biological tissue the course of clearance has been described from the amount retained in the lung after a certain time In other investigations in which data have been collected concerning the retention at different times a simple exponential function or a number of such functions have been fitted to the observations of lung clearance notwithstanding differences in aerosol materials animals and measurement techniques (1 7 8 62 65 73 98)

The majority of these studies have been confined to the later phases of lung clearance Only a few reports have been published concerning the initial rapid phase The difficulties experienced in interpreting this initial phase have probably been due to the distance dependence of the fixed detector system used It has often been found for instance that the measured values increase during the first hour or hours after exposure (1) possibly due to redistribution of the particles in the lungs

Although exponential functions make it easier to find biological explanations for the course of lung clearance there is no evidence to show that such functions represent the best mathematical models for describing the course of lung clearance for solid particles except in cases where the relevant mechanisms display an exponential course It has been shown for instance that the number of free phagocytes in the lungs is directly correlated to the retention of particles and that both parameters display an exponential elimination (65) Since the mechanisms involved in clearance are superimposed the various terms in an exponential function cannot be automatically ascribed to specific clearance mechanisms

An adequate test of different mathematical models accommodation of clearance data requires a large number of readings for the retention at different times separated—particularly during the initial phase—by only short intervals Frequent observations during the first hour or hours of clearance are also necessary owing to the rapid course of this phase in order to calculate the initial retention by extrapolation to the end of the exposure period This means that the mathematical function applied will be of decisive importance for the evaluation of the initial deposition

The observations obtained from the lung clearance studies presented in Chapter 7 were derived by the procedure of measuring the height of the scan curves They were corrected for the relevant sensitivity of the rate meter the physical decay of the isotopes and (in the di disperse studies) for the effect of superimposition of the energy spectrum of Sc^{46} in the Au^{198} channel in the gamma spectrometer (achieved automatically with the apparatus employed in the studies presented here) The mathematical model $Y = A\ e^{\alpha t} + B\ e^{\beta t}$ was fitted to these correced data expressed in arbitrary units, with the help of an iterative adjustment programme in the computer

The whole body measurements which started after or, in some cases simultaneously with the last measurements by profile scanning were fitted to a simple exponential function, partly in order to evaluate clearance during this period and partly so that these data could be converted into a common scale for profile scanning and whole body measurement In the latter case the sum of two exponential functions was fitted to the combined observations in order to elucidate the method s ability to describe the entire course of clearance The initial value obtained from this mathematical model was set at 100 % and all the observations were standardised to this figure

An example of the mathematical model s approximation to the observations for profile scanning measurements of lung clearance up to 150 hours is given in Fig 47 for 3 and 6 μm monodisperse polystyrene particles The course of clearance is shown on a linear scale to make it easier to observe the differences in the clearance of the two sizes of particle

Fig 48 reproduces the fit of the mathematical model for observations obtained during the first three hours after exposure to the above mentioned di disperse aerosol and Fig 49 does the same for the interval 0—1 000 hours with profile scanning and whole body measurement Fig 50 shows the same clearance course as in Fig 49 on a semi logarithmic scale in order to illustrate the exponential character of clearance

In many cases the first observation is off the regression line of the rapid phase of clearance No statistical evaluation can be made on the basis of these isolated values In the light of studies concerning the rate at

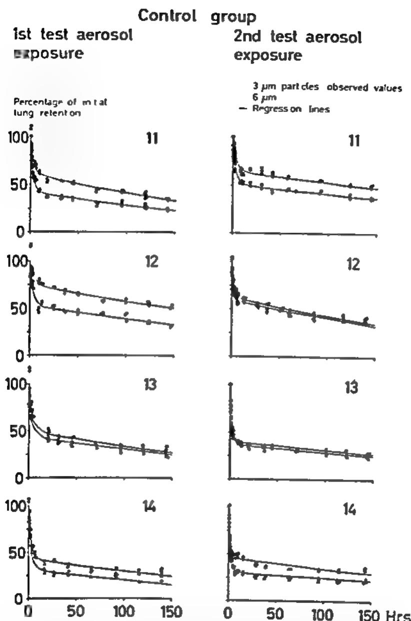


Fig 47 The simultaneous lung clearance of 3 and 6 µm polystyrene particles The values up to 150 hours after exposure to the particles were obtained with profile scanning apparatus no 4 The sum of two exponential functions was fitted to the observed values and the regression lines are shown. The figures in the diagram refer to the number of the rabbits in the experiment presented in Chapter 7 The interval between the two exposures was ca. 8 weeks

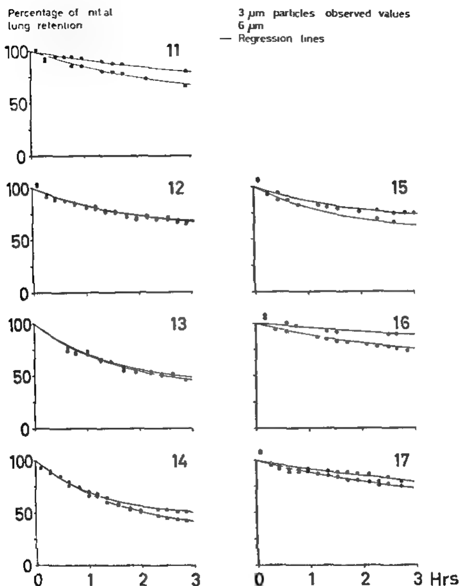


Fig 48 The simultaneous lung clearance of 3 and 6 µm polystyrene particles during the first three hours after exposure to the aerosol Note that the first plot in nearly all the experiments lies above the regression line The figures in the diagram refer to the number of the rabbits in the experiment presented in Chapter 7

Control group

1st test aerosol exposure

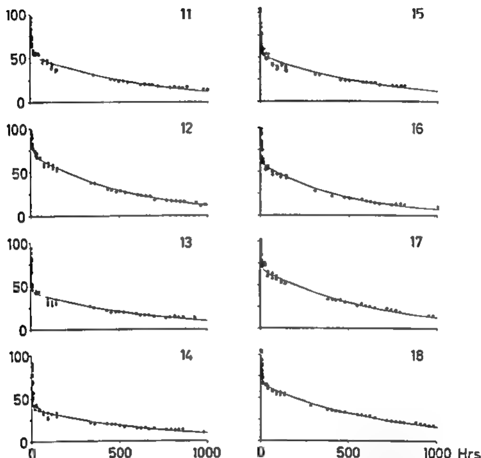


Fig 49 Lung clearance of 3 μm polystyrene particles The lung retentions were recorded by profile scanning and whole body measurements The regression lines (the sum of two exponential functions) are shown The figures in the diagrams refer to the same group of animals as in Figs 47 and 48

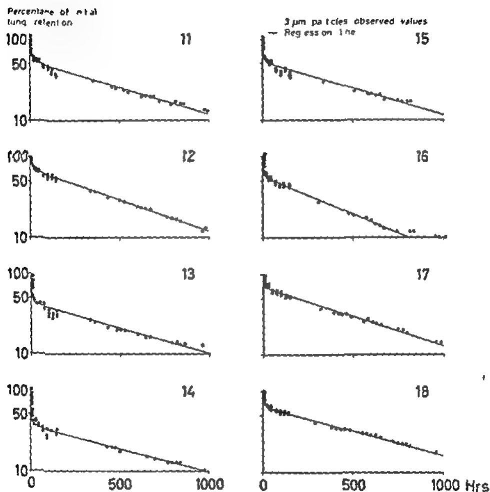


Fig 50 The same data as in Fig 49 plotted on a semilogarithmic scale

which secretion and particles are transported in the trachea (84) it is reasonable to suppose that this is an indication of a very rapid clearance (a half life of some 10 minutes) connected with matter deposited in the trachea. Each observation lasts 5—10 minutes in profile scanning and consequently the technique cannot be used to study this phenomenon in greater detail

By fitting the same mathematical model to all the clearance data a uniform basis is created for assessing these provided they are comparable with respect to the time of the first the frequency of subsequent and the duration of the observations

One of the advantages of fitting mathematical models to clearance data is that the constants included in the functions can be used for statistical processing The exponential functions also make it possible to compare the rates of elimination and the percentage of the total clearance process in the various phases of clearance

Discussion

The investigations presented here show that profile scanning as a means of measuring the retention of radioactively tagged aerosols in the lungs is a suitable method for studying the lung clearance of such particles The use of two detectors placed on either side of the animal reduced the interference —possibly due to the redistribution of the particles in the lungs—that occurs in external measurement with unilateral recording of the radioactivity from tagged particles deposited in the lungs In all external methods for measuring lung clearance the results can be affected by interference from transport of the substance studied through the oesophagus In this respect, profile scanning has the advantage that this transport can be observed In keeping with all other methods for external measurement profile scanning cannot however be used to detect translocation or storage of the substance in the lymphatic system in the thorax and consequently one can only study transport from the lungs as a whole

The profile scan curves can be evaluated planimetrically as described above or by a relatively simple procedure by measuring the height of the lung scan curve The latter technique is less time consuming than the former To describe the process of lung clearance from the data obtained, one can estimate either the time after exposure at which different levels of retention are reached the half life of total clearance or its slower phase This procedure however may introduce indeterminable errors particularly if the data display a large spread The course of clearance has therefore been visualised by fitting various mathematical models to the data for calculating the regression line The choice of mathematical model is decisive for the interpretation of the expression obtained for the clearance process As mentioned under "Mathematical models" a common method is to fit the sum of two or more exponential functions to the observed values This tradition has been followed in the present study although there is no definite evidence to show that these models give the best picture of the clearance function

CHAPTER 7

APPLICATION OF THE METHODS

The methods reported in previous chapters have been used to determine lung clearance in rabbits Since the original report by Friberg & Holma in 1961 (36), the methods for studying lung clearance have undergone continuous development as discussed in previous chapters The results of the earlier investigations will not be reported in detail, some have been presented in previous chapters The most advanced techniques have been used to determine lung clearance in normal and SO_2 exposed rabbits Sulphur dioxide was used for the study because of its significance as an industrial pollutant and also because it has featured so prominently in the discussion concerning air pollution in general

Experimental conditions

The experiments were conducted with 14 male rabbits[1] (weight approximately 3 5 kg) divided into two groups of seven animals each The experimental set up is shown in Fig 51

The rabbit s body temperatures and weights were monitered daily approximately one week prior to the start of the experiments to exclude sick animals

The production of the di disperse test aerosol (6 and 3 μm polystyrene particles tagged with Au^{198} and Sc^{46} respectively), the exposure technique and the measurement of lung clearance were performed as described in the preceding chapters The duration of exposure to the test aerosol varied between 5 and 10 minutes In order to evaluate the particle characteristics of the test aerosol, a certain volume of air in front of the animal s mouth was collected on millipore filters during exposure These filters were then analysed for the presence of particle aggregates by examination with the light microscope The total incidence of particle aggregates was found to be ca 5 % and ca 3 % for the 6 μm and 3 μm particles respectively

Two weeks after the experimental and control groups had been exposed to the test aerosol the former group was exposed to 10 ppm SO_2 for 16 hours a day in the chamber illustrated in Fig 52 10 % SO_2 from a cylinder was diluted with air to 10 ppm in a *special mixing chamber before the intake to* the exposure chamber The content of SO_2 in the latter was checked conductometrically with 2 hourly samples throughout exposure These analyses showed a mean of 10 ppm $\pm$ 1 ppm The distribution of the sulphur dioxide content in the chamber displayed no appreciable concentration gradient During the experiments the air temperature of the

[1] The experiments were originally designed for 16 rabbits divided into two groups of 8 animals each However one rabbit in the group exposed to SO_2 died in connection with anaesthesia prior to re exposure to the test aerosol Moreover one of the rabbits in the control group developed paralysis in a hind leg and was sacrificed An autopsy performed at the National Veterinary Insitute showed "degeneration of medullary sheath in spinal chord and peripheral nerves The temperature and weight of the animals were recorded during the experiments and found to be normal

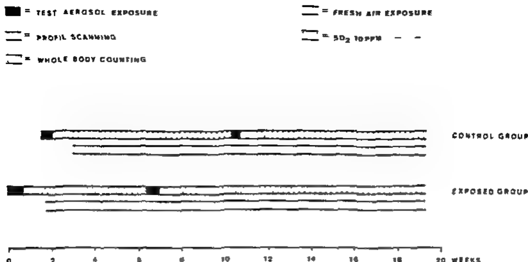


Fig 31 Experimental design for the study of lung clearance in normal and SO exposed rabbits

chamber was ca 20°C and the relative humidity ca 20—30 % The control group of animals was kept in a similar exposure chamber and exposed to fresh air under corresponding conditions and times

The experimental and control animals were re exposed to the di disperse test aerosol after about six and eight weeks respectively

Profile scanning performed with apparatus no 4 (cf Chapter 5) was started 1—2 minutes after the end of exposure to the test aerosol The first lung fractions on the profile curves were obtained after 5—10 minutes Scanning was then repeated every tenth to fifteenth minute during the next 3 or 4 hours and subsequently at intervals of 10 hours during the next 2 to 3 days and once a day thereafter Once the initial phase was over three scans were performed in succession on each occasion The profile scanning continued for approximately two weeks with some of the rabbits Others were only observed for about one week because they had a lower initial retention of Au^{198} (cf Tables 3—6 in the Appendix) This short observation period applies to the scannings after the first exposure to the test aerosol In order to obtain uniform observation periods for all the rabbits the results are based on a period of 6 days in all cases

Whole-body measurements were started at the time when profile scanning showed that there was no appreciable radioactivity in the gastrointestinal tract As a rule this occurred 5—10 days after exposure to the test aerosol (Fig 42) Only the 3 μm particles tagged with Sc^{46} were studied with the whole body counter because the half life of Au^{198} made it impossible to study the 6 μm particles which were tagged with this isotope Lung clearance of the 3 μm particles was followed with this technique for approximately 6 weeks In order to establish a uniform basis for the different animals an observation period of four weeks has been included in calculating the results

The sum of two exponential functions was fitted to the data from the profile scanning measurements using a computer and an iterative least squares estimation programme

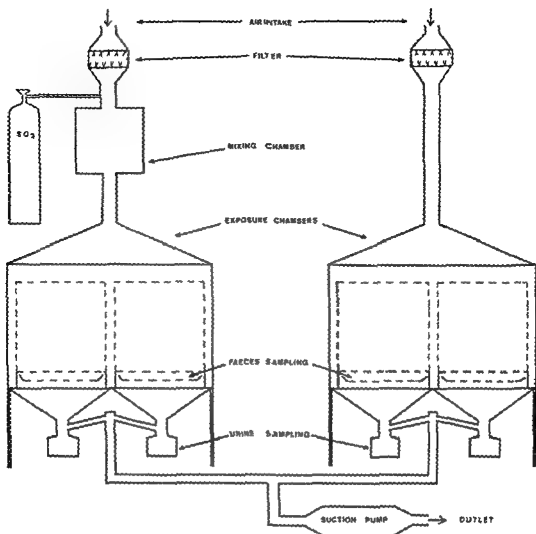


Fig. 32 Diagram of the exposure chambers

The results from the whole body measurements were treated in two ways. First they were combined with the data from the profile scanning measurements as described in the chapter on mathematical models and, second they were treated separately by fitting a simple exponential function to the observed values. Since it was desired to study whether the interjection of exposure to SO_2 affects the slower phase of clearance this exposure was started at the time of the transfer from profile scanning to whole body measurement. For this reason, the whole body measurements were evaluated separately as described above.

In the following presentation of the results the suffixes $_p$ and $_w$ are used in connection with the regression parameters in the expression $Y = A \; e^{\alpha t} + B \; e^{\beta t}$ to refer to profile scanning measurements and whole body measurements respectively. In the mathematical model Y is the fraction

of the intercept value remaining at given times (t) in hours and e is the natural logarithm base A and B are the percent of the intercept value λ (t = o) for the first and second phase of clearance respectively The α and β constants refer to the slopes of the first and second phase respectively The results of the investigation will be discussed under the following headings

1 The course of clearance and its different phases
2 Intra and inter individual variations
3 Significance of particle size
   a) Clearance of different sizes of particle
   b) Correlation between regression parameters for differen particle sizes
4 Correlation between different phases of clearance
5 Clearance after exposure to sulphur dioxide

Results and comments

The two phases of lung clearance as evaluated from the profile scanning measurements are shown in Tables 5—8 The mean values for alfa coefficients are shown in Table 5 the beta coefficients in Table 6 and the intercept of the second phase (B) with the y axis in Table 7 Data concerning beta coefficients obtained from whole body measurements are given in Table 8

The dose of radiation received by the lung tissue assuming complete absorbtion of the irradiation from the beta decay averaged approximately 18 rad the highest mean for a group being 55 rad.

The course of clearance and its different phases

The course of clearance and its different phases are illustrated in Fig 53 *A* for the two phases for 3 μm particles as determined by profile scanning and in Fig 53 *B* for the slower phase using the whole body counting technique The individual curves were calculated mathematically (see Chapter 6) from the empirical data of the control group after the first exposure to the aerosol It will be seen that clearance is characterised by a rapid initial phase and a secondary slower phase The course of clearance demonstrated largely agrees with earlier results published by the author (53 54) as well as with the results of other researchers referred to in Chapter 6 where mathematical models are discussed

The half lives of the two phases are illustrated in Fig 54 in which the slopes for the different phases relate to the 3 μm particles in the control group after the first exposure to the test aerosol Here again one sees a rapid initial phase and a slower second phase moreover the slope of the slower phase as calculated from whole body measurements is somewhat lower than that obtained by profile scanning probably due in part to differences in the length of observation periods The intercept data given in Table 7 show that the second phase is responsible for the greatest proportion of the total elimination of the smaller particles while the corresponding elimination of the larger particles is less consequently the first phase eliminates more of the larger particles than of the smaller ones This may be taken as indicating that the larger particles are deposited at the tracheobronchial level to a greater extent than the smaller ones

Further investigations are required before one can establish with certainty which clearance mechanisms are chiefly responsible for

TABLE 5 Means of α_p coefficients obtained from profile scanning measurements

Exposure	Control group		Experimental group	
	3 μm	6 μm	3 μm	6 μm
I	0 47	0 49	0 32	0 42
II	0 47	0 51	0 46	0 49
[illegible]	±0 07		±0 06	

S = Average standard error of individual mean

TABLE 6 Means of β_p coefficients obtained from profile scanning measurements

Exposure	Control group		Experimental group	
	3 μm	6 μm	3 μm	6 μm
I	0 0037	0 0041	0 0036	0 0048
II	0 0027	0 0029	0 0041	0 0054
S	±0 0003		±0 0005	

S = Average standard error of individual mean

TABLE 7 Means of B (the intercept for the slower phase) obtained from profile scanning measurements

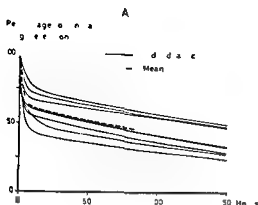
Exposure	Control group		Experimental group	
	3 μm	6 μm	3 μm	6 μm
I	59	40	64	37
II	58	51	63	42
S	± 4		± 5	

S = Average standard error of individual mean

TABLE 8 Means of β_w coefficients obtained from whole body measurements

Exposure	Control group	Experimental group
	3 μm	3 μm
I	0 0012	0 0017
II	0 0013	0 0016
S	±0 0001	±0 0001

S = Average standard error of individual mean



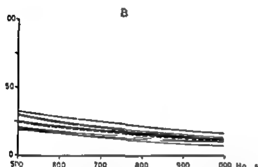
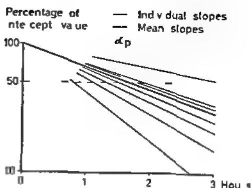
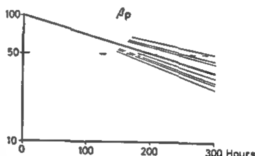


Fig 53 (*A*) Mean and individual lung clearance curves for the control rabbits after the first aerosol exposure obtained by profile scanning (*B*) Mean and individual curves of the slower phase of lung clearance for the same animals obtained by whole body measurement





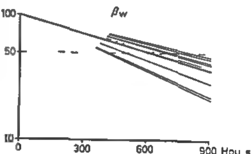


Fig 54 Mean and individual slopes for the different phases of lung clearance in the control rabbits after the first test aerosol exposure Upper diagram The first phase obtained by profile scanning Middle diagram The second phase obtained by profile scanning Lower diagram The second phase obtained by whole body measurement Note the differences in the time scales

the different phases However since the rate of mucociliary transport is relatively rapid (84) this should be the clearance mechanism that is chiefly responsible for the rapid initial elimination of the aerosols deposited in the ciliated parts of the respiratory tract and thus represented by the intercept (A) of the first term in the mathematical function used The study of retention (pp 24—31) supports this assumption as it indicates that the alveolar clearance mechanisms do not play a significant part in the initial phase thus the retention recorded in the peripheral parts of the lung did not change appreciably during the first 72 hours

It is more difficult to relate the slower phase of clearance to particular mechanisms even if as a consequence of the discussion of the first phase these should be found in nonciliated regions Further experimental studies are required for this as well as to

determine whether Brieger (15) is correct in stating that "the early rapid clearance is not limited to removal of bronchial deposits as Collet has suggested"

Intra and inter individual variations

It will be seen from the data presented above that there is considerable variation between individual animals It would be interesting to know whether this variation is essentially conditioned by characteristics of the individual expressed as a certain stability in the course of clearance at different times This individual consistency can be estimated by analysing the relationship between the "inter rabbit" and the "intra rabbit" variability It should however be noted that in this type of experimental design the "intra rabbit variability" cannot be distinguished from the experimental error Under the circumstances it is nevertheless the closest measure obtainable of the "intra rabbit variation" In the case of the alpha coefficient for the experimental group the "inter rabbit variation was greater ($p < 0\ 001$) than the estimated experimental error For the alpha constant in the control group the relationship bordered on significance ($0\ 10 > p > 0\ 05$) In the case of the beta coefficient the difference was significant in the experimental group ($0\ 05 > p > 0\ 01$) but not in the control group Although the results were not significant throughout it can be considered that the present experimental design is preferable to a direct inter group comparison without allowing for the preexposure values of each group

In a previous study in rabbits with 4 μm n octadecanol particles the author (53) was not able to find any larger inter than intra individual variations This may have been due to the larger initial retention (mg) in these rabbits In studies concerning the "alveolar clearance of submicronic iron oxide particles, Gibb & Morrow (38) found good reproducibility of the mean biological half time in a group of six dogs re exposed 6 months after the first exposure The standard deviation for data from the first exposure was 5 % and for data from the second exposure 25 %

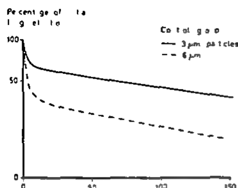
Significance of particle size

Clearance of different sizes of particle

The difference in the course of clearance between 3 and 6 μm particles obtained by the profile scanning technique is shown in Fig 55 which gives the regression line for the mean values of the control group and the exposed group before exposure to SO_2

An analysis of variance was performed on the combined results for the experimental and control animals The differences in the alpha constant bordered on significance ($0\ 10 > p > 0\ 05$) and those in the beta constant were significant ($0\ 05 > p > 0\ 01$)

The results show that the difference observed is partly ascribable to differences in the rate of clearance for the different sizes of particles (as expressed in differences in alpha and beta constants) but that other factors are also involved Thus the fact that the initial clearance of 6 μm particles is greater than that of 3 μm particles seems to be largely ascribable to a larger amount of the former being deposited in the tracheo bronchial parts of the respiratory tract which agrees with the results reported in Chapter 3 As far as the initial phase is concerned the result agrees with reports that there is no difference in the rate of transport of different sized particles applied directly to the trachea (4) The differences in lung clearance patterns for the particles used was to be



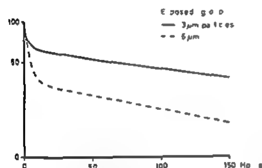


Fig 55 The simultaneous lung clearance of 3 and 6 μm polystyrene particles during the first 150 hours after exposure Upper diagram Regression curves of means for the animals in the control group Lower diagram Regression curves of means for the animals in the experimental group The fi gure shows the difference in lung clearance be tween the two particle sizes

expected in the light of data already referred to from the literature (cf p 11) although except by the author (54) no investigations had been made earlier either with mono disperse aerosols or simultaneously in one and the same animal with two different monodisperse particle sizes

Correlation between regression parameters for different particle sizes

In spite of differences in deposition and clearance rates between the different sized particles it has already been noted that there is reason to suppose that the same mecha nisms in principle are responsible for the clearance of both sizes If this is the case one would expect to find a correlation be tween α_p for 3 and 6 μm as well as between β_p for the two sizes in the same animal

The correlations obtained for α_p and β_p are shown in Fig 56 *A* and *B* The product moment correlation coefficient for α_p was 0 77 ($p < 0\ 001$) in a one sided test for β_p it was 0 49 ($0\ 05 > p > 0\ 01$)

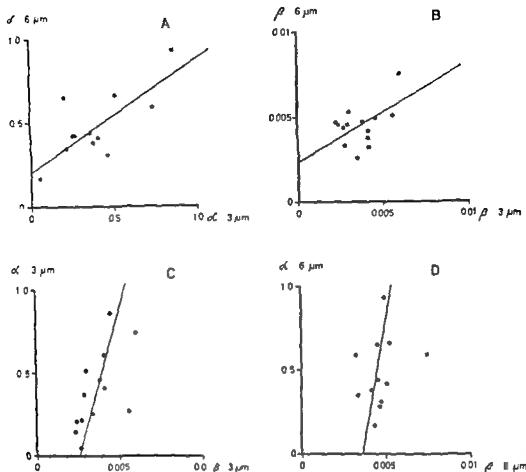
These correlations show that there is good agreement between the two sizes concerning the rate of both the initial and the later phase of clearance

Correlation between different phases of clearance

A further question is whether or not there is a correlation between the rapid and slower phases of clearance as expressed in α_p and β_p coefficients The existence of such a rela tionship would indicate the presence of a factor common to both clearance mecha nisms The product moment correlation be tween α_p and β_p was 0 61 ($0\ 05 > p > 0\ 01$) for 3 μm particles in a two sided test the correlation was not significant ($r = 0\ 26$) for 6 μm particles (Fig 56 *C* and *D*)

Clearance after exposure to sulphur dioxide

The situation before and after exposure to SO_2 is illustrated in Fig 57 which shows the mean curves of lung clearance for the 3 and 6 μm particles obtained by the profile scanning for both the control and experimen tal groups The pre and postexposure data for each animal were compared to determine the effect of SO_2 exposure The differences between these data were then subjected to an



F g 6 Compar son of data obtained after the first test aerosol exposure in the on and exper mental groups The correlation was studied between (*A*) the ■ ft ts for 6 and 3 μm polystyrene particles ($r = 0\ 77$ $p < 0\ 001$) (*B*) the ef nts for the same part cle sizes ($r = 0\ 49$ $0\ 05 > p > 0\ 01$) (*C*) the α c ents for the 3 μm part cles ($r = 0\ 061$ $0\ 05 > p > 0\ 01$) and (*D*) 3 ff cients for the 6 μm particles ($r = 0\ 26$)

analysis o he two particle sizes being te ependently A comparison between ol and experimental groups showed c did not differ with respect to the α o s art for both sized particles A significant differ nce ($0\ 05 > p > 0\ 01$) was demonstrated for the β_p in the clearance of 3 μm part cles (Fig 58) A similar trend was found for the β_p of the 6 μm particles The differences found were ascribable in part ■ an increase in the β_p constant in the exposed group and partly to a decrease in the control group It is clear that a knowledge of changes in the control group was essential in determining the effects of SO_2 in the experi mental group This emphasizes the necessity of studying both control and experimental groups before as well ■ after exposure

An analysis of variation of the β_w constant for the control and experimental groups showed a significant difference for the 3 μm particles during the first 30 days of exposure

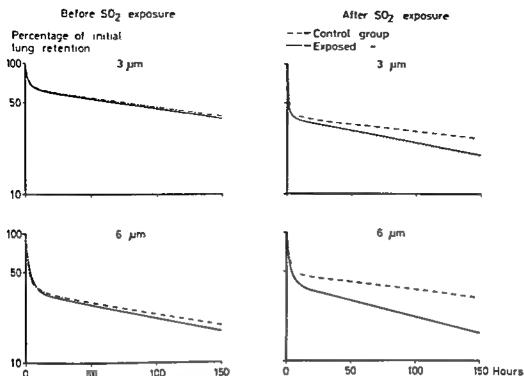


Fig 57 The regression curves of mean lung clearance values for 3 and 6 µm polystyrene particles (control and experimental groups) before and after SO_2 exposure

but no such differences could be found for the period between the 40th and the 70th day of exposure to SO_2 (Fig 59)

The study shows that SO_2 (10 ppm—16 hours per day) accelerates the slower phase of lung clearance of 3 µm polystyrene particles during the first 6 weeks This effect diminished during the next 4 weeks Although the mechanisms of lung clearance affected by the SO_2 exposure remain obscure the initially increased rate of secondary phase of clearance of 3 µm and 6 µm particles indicates that the effect is more pronounced at the pulmonary level As increased secretion is an early manifestation of inflammation and it has been demonstrated that pulmonary oedema in inflamed lung increases particle removal (47) such a mechanism may be responsible for the increased rate of lung clearance reported here Subsequent inflammatory changes i e epithelial proliferation with crypt formation and changes in the characteristics of the secretion have been demonstrated by Dalhamn to reduce the rate of secretory transport (25) These changes could explain the decrease in clearance observed during the 6—10 week period

The changes in lung clearance observed here are essentially in agreement with those reported by Vyskocil *et al* (102) which showed a significant increase in the rate of lung clearance of quartz dust in rabbits after exposure for one month to 50 % sulphuric acid aerosol and a decrease (though not significant) after exposure for one year The histological effects were more pronounced

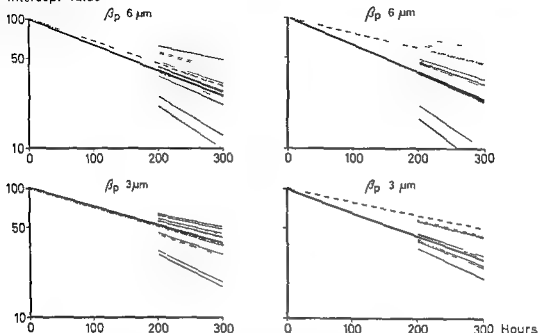


Fig 58 Mean and individual slopes for the second phase of lung clearance of 6 and 3 μm polystyrene particles (control and experimental groups) obtained from the profile scannings before and after SO_2 exposure

at alveolar level than in the trachea after one year Schlipkoter & Brockman (88) also used the classical method on sacrificed animals and found that the elimination of SiO_2 dust was decreased in rats which were exposed to SO_2 gas for one month prior to and one month after the dust exposure The different result may be explained by differences in the animals and particle materials as well as by different intensities of exposure — continuous contra 16 hours a day

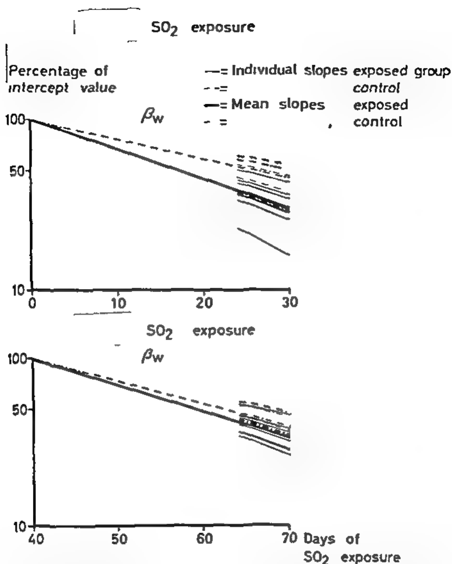


Fig 59 Mean and individual slopes for the second phase of lung clarance of 3 μm polystyrene particles (control and experimental groups) obtained from the whole-body countings during the first 30 days and between the 40th and 70th day of SO_2 exposure

CHAPTER 8

GENERAL DISCUSSION

The investigations presented in this study show that measurement of the retention of radioactively tagged aerosols in the lungs by means of profile scanning can be applied to the study of the clearance of such particles from the lung The area of the lung scan curves evaluated planimetrically in the manner described or by measurement of the peak height of the lung fraction can be used to determine the amount of retention of the particles in the lung

One of the advantages of profile scanning is that compared with other techniques for external measurement it is less affected by changes in position of the activity within the volume being studied Moreover, the method makes it possible to establish the time when the lung becomes the only organ containing a major proportion of the substance being investigated and hence the stage at which one is justified in measuring lung clearance by means of whole body counting The two methods therefore make it possible to follow lung clearance from its initial phase through several months

Translocation to or storage of a radioactive substance in the lymphatic system of the lungs cannot be detected by external measurement which can only be used to study the transport from the whole of the thorax. Although this limitation does exist there is no reason to expect any significant storage in the lymphatics of the lung unless the particles in question are highly cytotoxic.

The condensation and spinning disc methods used to produce monodisperse radioactively tagged (Au^{198} and Sc^{46}) particles of n octadecanol and polystyrene have been shown to be suitable for lung clearance studies with external measuring techniques

The observed difference in the clearance of the different sized particles (3 and 6 μm) seems to be chiefly ascribable to differences in the depth of deposition In part the difference was due to the fact that the larger particles were eliminated more during the initial phase of clearance because they were deposited to a greater extent on the ciliated epithelium. But the difference was also due to the rate of clearance for the secondary phase being faster for the larger particles than for the smaller ones A similar though not definitely significant difference was also found for the initial phase Different clearance rates for 6 and 3 μm particles were demonstrated both by the clearance studies and by the study of the retention of monodisperse particles at different levels in the lung The latter study showed that the larger particles were deposited more in the trachea and bronchi than the smaller ones The findings also indicated that the amount of initial retention of both particle sizes in the pulmonary region remains essentially unchanged during the first 72 hours The rate of elimination for both sized particles increased successively from the periphery of the lung towards the trachea, the increase being greater for the larger particles both quantitatively and on a percentage basis

The different rates of clearance for 3 and

6 μm particles during the slower phase cannot be explained by the studies performed though the study of the phagocytosis of polystyrene particles by lung macrophages in vitro indicated that the 3 μm particles were phagocytosed to a greater extent than the 6 μm particles This may be an indication that the smaller particles are affected by phagocytosis at the non ciliated parts of the lung in a way that reduces their clearance rates One possibility for this is that the alveolar lining cells per se are phagocytic

The intra and inter individual variations demonstrated emphasize the advantage of evaluating the clearance of different particle sizes simultaneously in the same animal since this keeps both the time factor and the biological variations under control. It also points to the desirability of making comparative experiments on both control and experimental animals before as well as after exposure to the substance being investigated This design used to study the effect of SO_2 (10 ppm—16 hours a day during ca 10 weeks) on lung clearance of 6 and 3 μm particles showed no effect on the initial phase of clearance after 5 weeks of SO_2 exposure but an accelerating effect on the slower phase for the 3 and the 6 μm particles during the first 6 weeks of SO_2 exposure This effect on the slower phase was diminished by exposure beyond 6 weeks The results suggest that the initial effects of SO_2 on lung clearance are more pronounced at the pulmonary than the tracheo bronchial level

An experimental design using two particle sizes one being more tracheo-bronchial and the other more pulmonary in deposition can thus be used to determine the site of action of the agents on lung clearance Furthermore the use of two different tagged monodisperse particles e g one highly and the other slightly susceptible to phagocytosis can be of value for studying the relative importance of various mechanisms involved in total lung clearance Evaluating the changes in clearance of different test aerosols by stimulating or blocking individual components might provide valuable complementary data in such studies

GENERAL DISCUSSION

The investigations presented in this study show that measurement of the retention of radioactively tagged aerosols in the lungs by means of profile scanning can be applied to the study of the clearance of such particles from the lung. The area of the lung scan curves evaluated planimetrically in the manner described or by measurement of the peak height of the lung fraction can be used to determine the amount of retention of the particles in the lung.

One of the advantages of profile scanning is that, compared with other techniques for external measurement, it is less affected by changes in position of the activity within the volume being studied. Moreover, the method makes it possible to establish the time when the lung becomes the only organ containing a major proportion of the substance being investigated and hence the stage at which one is justified in measuring lung clearance by means of whole body counting. The two methods therefore make it possible to follow lung clearance from its initial phase through several months.

Translocation to or storage of a radioactive substance in the lymphatic system of the lungs cannot be detected by external measurement, which can only be used to study the transport from the whole of the thorax. Although this limitation does exist, there is no reason to expect any significant storage in the lymphatics of the lung unless the particles in question are highly cytotoxic.

The condensation and spinning disc methods used to produce monodisperse radioactively tagged (Au^{198} and Sc^{46}) particles of n-octadecanol and polystyrene have been shown to be suitable for lung clearance studies with external measuring techniques.

The observed difference in the clearance of the different sized particles (3 and 6 μm) seems to be chiefly ascribable to differences in the depth of deposition. In part the difference was due to the fact that the larger particles were eliminated more during the initial phase of clearance because they were deposited to a greater extent on the ciliated epithelium. But the difference was also due to the rate of clearance for the secondary phase being faster for the larger particles than for the smaller ones. A similar though not definitely significant difference was also found for the initial phase. Different clearance rates for 6 and 3 μm particles were demonstrated both by the clearance studies and by the study of the retention of monodisperse particles at different levels in the lung. The latter study showed that the larger particles were deposited more in the trachea and bronchi than the smaller ones. The findings also indicated that the amount of initial retention of both particle sizes in the pulmonary region remains essentially unchanged during the first 72 hours. The rate of elimination for both sized particles increased successively from the periphery of the lung towards the trachea, the increase being greater for the larger particles both quantitatively and on a percentage basis.

The different rates of clearance for 3 and

ticles did not differ significantly from each other The second slower phase however was faster for the larger particles For the two particle sizes there was a correlation between their rates of clearance in the first and second phase A correlation was also found between the rates of clearance in the first and second clearance phase for the 3 μm particles

The question whether the lung clearance rate was influenced by individual components was studied by comparing the intra and interindividual variations by means of an analysis of variance

The results showed that for the two clearance phases the interindividual variations were significantly greater than the estimated experimental error in one of the groups In the other group this relationship bordered on significance for the first clearance phase while no significance was found for the second phase

The effect of SO_2 (10 ppm—16 hours a day during ca 10 weeks) on lung clearance of a di disperse aerosol (6 and 3 μm particles) was evaluated by comparing the exposed with the control group No effect was seen on the first phase during 5 weeks of SO_2 exposure An accelerating effect was however observed during the first six weeks of the slower phase of the 3 μm particles and the 6 μm particles Prolonged exposure—more than 6 weeks—diminished this effect on the slower phase

In order to further elucidate the possible difference in retention between the different particle sizes the retentions of the monodisperse 3 and 6 μm particle sizes were compared at different levels of the lung and at different times after exposure using a new technique based on scaler measurements on selected specimens cut out from formaline fixed and air dried lung preparations The findings indicate that the initial retentions of both particle sizes in the alveoli remain essentially unchanged during the first 72 hours The larger particles were found to be deposited more in trachea and bronchi than the smaller ones This study also demonstrates that the larger particles were eliminated more rapidly than the smaller ones from the lung The rate of elimination for both sized particles increased successively from the periphery of the lung towards the trachea the increase being greater for the larger particles both quantitatively and on a percentage basis

In order to study whether the phagocytosis was notably different for different particle sizes—and also to what extent the phagocytosis of the polystyrene particles used differed from that of carbon particles—an experiment was performed according to the in vitro method introduced by Myrvik *et al* measuring the extent to which [redacted] 1 5 μm carbon particles as well as 1 5 3 0 and 6 0 μm polystyrene particles underwent phagocytosis by rabbit lung macrophages As for the particle sizes the results indicate that the smaller particles were more susceptible to phagocytosis than the larger ones A comparison with carbon particles showed that these undergo phagocytosis to a considerably greater extent than do particles of polystyrene of equivalent size

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APPENDIX

TABLE 1 Phagocytosis of polystyrene particles of different sizes

Experiment Code symbols given in Table 7	Phago cytes per sq mm	Phagocyte particles			Phagocytes that performed phago cytosis (%)	Particles phagocyto sed in 1 5 hours (%)		
		1 5 µm	3 µm	6 µm	1 5 hrs	1 5 µm	3 µm	6 µm
A II	850		1 8		20		2 7	
	850		1 10		22		2 6	
	400		1 8		30		4 8	
	1 280		1 5		24		5 4	
Mean	845		1 78		24		3 9	
A III	850	1 2		1 5	37	11 6		8 5
	850	1 2		1 5	33	13 6		4 5
	400	1 4		1 8	44	8 6		3 9
Mean	700	1 25		1 60	[illegible]	11 3		5 6
A IV	850	1 2	1 5	1 4	35	6 4	2 6	4 9
	850	1 2	1 7	1 4	40	13 5	2 2	2 0
	400	1 3	1 4	1 5	39	7 9	4 6	3 8
Mean	700	1 73	1 53	1 43	38	9 3	3 1	3 6
B II	1 680		1 8		25		4 7	
	1 250		1 6		36		11 0	
	1 080		1 4		38		10 7	
Mean	1 337		1 60		33		8 8	
B III	1 450	1 2		1 2	27	14 2		5 4
	1 450	1 2		1 2	29	18 4		6 3
	1 680	1 1		1 4	31	20 1		7 5
	850	1 5		1 5	29	7 4		5 0
	1 080	1 3		1 2	37	17 0		4 2
Mean	1 306	1 76		1 30	31	15 4		5 7

Experiment Code symbols given in Table 2	Phagocytes per sq mm	Phagocyte : particles			Phagocytes that performed phagocytosis (%)	Particles : phagocytosed in 1 5 hours (%)		
		1 5 μm	3 μm	6 μm	1 5 hrs	1 5 μm	3 μm	6 μm
B IV	1 450	1 2	1 6	1 2	35	11 9	6 2	2 1
	1 450	1 2	1 6	1 2	46	18 8	6 1	5 6
	1 680	1 4	1 5	1 3	35	7 4	3 5	3 9
	1 080	1 5	1 5	1 3	39	8 9	3 4	3 4
	1 250	1 2	1 6	1 4	44	12 7	8 1	4 8
Mean	1 386	1 3 0	1 5 6	1 2 9	40	11 9	5 5	4 0
C II	1 190		1 5		23		6 8	
	950		1 7		22		5 0	
	470		1 12		36		4 4	
Mean	870		1 8 0		27		5 4	
C III	950	1 4		1 4	30	12 9		2 2
	470	1 6		1 8	39	7 7		2 4
	1 190	1 2		1 3	44	22 9		9 8
	950	1 7		1 6	30	6 6		0 6
Mean	890	1 4 8		1 5 3	36	12 5		3 8
C IV	1 190	1 2	1 2	1 3	24	7 7	5 7	1 4
	950	1 2	1 3	1 2	30	10 1	4 5	0 7
Mean	1 070	1 2	1 2 5	1 2 5	27	8 9	5 1	1 1
D IV	1 190	1 2	1 2	1 3	23	6 7	7 0	1 7
	950	1 2	1 3	1 5	35	12 4	6 2	1 2
	470	1 [illegible]	1 4	1 7	46	11 0	7 1	3 5
	1 680	1 3	1 4	1 4	45	13 0	3 8	2 5
Mean	1 073	1 2 3	1 3 3	1 4 8	37	10 8	6 0	2 2
E I*	1 [illegible]80	1 8			92			
	400	1 23			89			
	850	1 62			100			
	850	1 33			92			
	400	1 [illegible]8			98			
Mean	756	1 30 8			94 2			

* Particle size [illegible] 5—2 5 μm

TABLE 2 Code to Table 1 for particle sizes and combinations in the study of phagocytosis

A II = 3 μm	polystyrene particles untagged
A III = 1 5 and 6 μm	polystyrene particles untagged
A IV = 1 5 3 and 6 μm	polystyrene particles untagged
B II = 3 μm	polystyrene particles tagged with Sc
B III = 1 5 and 6 μm	polystyrene particles tagged with Au
B IV = { 3 μm	polystyrene particles tagged with Sc
{ 1 5 and 6 μm	polystyrene particles tagged with Au
C II = 3 μm	polystyrene particles tagged with Sc^{46}
C III = 1 5 and 6 μm	polystyrene particles tagged with Au^{198}
C IV = { 3 μm	polystyrene particles tagged with Sc^{46}
{ 1 5 and 6 μm	polystyrene particles tagged with Au^{198}
D IV = 1 5 3 and 6 μm	polystyrene particles tagged with Sc^{46}
E I = 0 5—2 5 μm	carbon particles

TABLE 3 Data concerning the lung clearance of 3 and 6 µm CMD polystyrene particles for the experimental group after the first test aerosol exposure

Rabbit no	Initial radioactivity (µc)		Initial retention (µg)		First phase of lung clearance				Second phase of lung clearance			
					% of initial retention (A)*		Half lives in hours		% of initial retention (B)*		Half lives in hours	
	Sc^{46}	Au^{198}	3 µm	6 µm	3 µm	6 µm	3 µm	6 µm	3 µm	6 µm	3 µm	6 µm
1	0 5	7 8	0 1	8 8	30 2	48 2	13 9	4 1	69 8	51 8	253	161
2	0 4	6 0	0 1	4 4	25 5	52 6	3 3	1 1	75 4	47 4	284	155
3	0 7	3 9	0 1	3 0	40 1	62 7	1 3	1 1	59 9	37 3	235	134
4	0 2	6 9	0 1	6 6	52 0	75 5	2 8	1 7	52 2	24 5	207	277
5	0 4	4 9	0 1	4 9	41 9	59 0	1 3	1 4	58 1	41 0	174	100
6	1 1	11 6	1 2	2 9	25 0	59 9	4 6	2 5	75 0	40 1	299	151
7	0 5	7 0	1 0	3 1	40 9	70 2	0 9	1 2	59 1	29 8	117	94
8	0 2	2 4	0 5	1 3	44 9	73 6	2 6	1 7	55 1	26 4	124	139
Mean	0 5	6 3	0 4	4 4	37 6	62 7	3 8	1 9	63 1	37 3	212	151

* A and B = Intercept for the first and second phase respectively

TABLE 4 *Data concerning the lung clearance of 3 and 6 μm CMD polystyrene particles for the control group after the first test aerosol exposure*

Rabbit no	Initial radioactivity (μc)		Initial retention (μg)		First phase of lung clearance				Second phase of lung clearance			
					% of initial retention (A)*		Half lives in hours		% of initial retention (B)*		Half lives in hours	
	Sc^{46}	Au^{198}	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm
11	1 2	19 9	0 7	31 7	37 7	57 2	1 8	1 8	6[illegible] 3	42 8	169	169
12	1 3	14 6	0 8	31 0	25 0	16 5	3 2	2 0	75 0	53 5	253	210
13	0 5	4 1	0 3	6 8	49 3	55 8	1 7	1 7	50 7	14 2	169	187
14	0 6	5 0	0 3	10 5	56 7	68 2	1 5	2 2	49 3	31 8	182	117
15	0 6	8 7	0 3	18 6	43 1	61 6	0 8	0 7	56 9	35 1	1.6	141
16	0 7	3 4	0 7	6 8	38 2	64 5	1 1	1 2	61 8	35 5	169	217
17	0 9	18 0	0 9	45 8	27 0	60 7	1 9	1 6	73 0	39 3	210	151
18	1 5	23 2	1 6	21 2	31 9	72 7	1 3	1 4	68 1	27 3	299	111
Mean	0 9	12 2	0 7	22 3	38 6	61 3	1 7	1 6	62 1	38 7	205	171

* A and B = Intercept for the first and second phase respectively

TABLE 5 Data concerning the lung clearance of 3 and 6 μm CMD polystyrene particles for the experimental group after the second test-aerosol exposure

Rabbit no	Initial radioactivity (μc)		Initial retention (μg)		First phase of lung clearance: % of initial retention (A)*		First phase of lung clearance: Half lives in hours		Second phase of lung clearance: % of initial retention (B)*		Second phase of lung clearance: Half lives in hours	
	Sc^{46}	Au^{198}	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm
1	1 3	18 9	1 0	32 7	29 8	46 5	9 9	6 3	71 2	53 5	118	[illegible]
2	1 0	5 7	0 8	10 3	41 0	51 5	0 8	1 4	56 0	49 5	152	198
3	0 8	19 9	0 4	12 2	[illegible]	63 4	1 1	1 5	73 9	36 6	2 0	169
4	1 3	[illegible]	1 5	16 0	25 0	42 6	13 9	1 5	75 0	37 1	169	139
5	—	—	—	—	—	—	—	—	—	—	—	—
6	1 9	[illegible]	0 9	17 3	55 0	90 2	5 8	2 8	45 0	19 8	273	143
7	[illegible]	19 0	2 1	3 1	50 6	62 3	0 7	0 5	49 1	37 7	131	91
8	1 7	19 7	1 9	31 8	27 6	59 3	3 0	2 5	72 1	41 7	155	173
Mean	1 4	18 1	1 3	18 1	36 7	57 8	5 0	2 4	63 3	42 2	161	140

* A and B = Intercept for the first and second phase respectively

TABLE 6 Data concerning the lung clearance of 3 and 6 μm CMD polystyrene particles for the control group after the second test aerosol exposure

Rabbit no	Initial radioactivity (μc)		Initial retention (μg)		First phase of lung clearance				Second phase of lung clearance			
					% of initial retention (A)*		Half lives in hours		% of initial retention (B)*		Half lives in hours	
	Sc^{46}	Au^{198}	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm	3 μm	6 μm
11	1 2	19 0	0 7	10 2	34 4	47 7	2 4	1 8	65 6	52 3	332	300
12	1 2	17 4	0 7	9 7	39 7	36 7	1 2	1 1	60 3	63 3	226	180
13	1 3	19 0	0 7	12 3	62 1	59 3	1 1	1 0	37 9	40 7	250	247
14	1 1	20 4	0 8	11 4	54 0	69 1	0 9	1 1	46 0	30 9	238	278
15	1 9	19 2	1 1	18 4	33 2	43 8	1 3	1 1	66 8	56 2	218	233
16	1 5	17 4	0 9	15 9	32 1	36 8	4 [illegible]	1 9	67 9	63 2	578	365
17	1 4	18 1	0 8	19 7	35 9	46 5	2 7	2 4	64 1	53 5	206	175
Mean	1 1	18 6	0 8	14 4	41 6	48 6	2 0	1 5	58 4	51 4	293	254

* A and B = Intercept for the first and second phase respectively

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SUPPLEMENTUM 474

ON EPIDEMIOLOGIC METHODS FOR RECORDING ISCHAEMIC HEART DISEASE

by

PER BJURULF, TORSTEN GARLIND AND
NILS H STERNBY

LUND 1967

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SUPPLEMENTUM 474

FROM THE DEPARTMENTS OF SOCIAL AND PREVENTIVE MEDICINE
(HEAD PROF G LINDGREN) AND PATHOLOGY (HEAD PROF F LINELL)
UNIVERSITY OF LUND GENERAL HOSPITAL, MALMÖ

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Contents

Introduction

The clinical picture of angina pectoris and myocardial infarction is often unequivocal. It has, however, recently been realized that a fair number of cases run an atypical course (2). Thus the pain regarded as characteristic may vary considerably in site and intensity, and in some instances there may be no symptoms at all (1, 2). This gives rise to problems in the recording of ischaemic heart disease (IHD) in epidemiological studies. For the uncharacteristic symptoms are sometimes difficult to distinguish from manifestations of other diseases of the chest and the upper part of the abdomen. Therefore, if such symptoms are accepted as evidence of IHD, it may result in other diseases to some extent being erroneously diagnosed as IHD.

These uncharacteristic symptoms were discussed by a study group appointed by WHO. It was decided by the group that "A diagnosis of angina pectoris based on the broad definitions employed in clinical medicine—which aims at the diagnosis of 100 % of cases—would cover too many borderline or atypical cases to be conducive to reproducible results in an epidemiological study" (5). This study group thus feels that the atypical cases should not be accepted. In various questions and problems to be elucidated by epidemiological studies it is of interest to have a method capable of separating off one group in which most of the individuals really have a given disease, e.g. IHD. The use of such a method, however, often implies that the group judged as not having IHD will include several cases with the disease. This, on the other hand, can have certain disadvantages when it is desired to separate off *also* a group as free as possible from IHD.

This stresses the importance of good knowledge of different qualities of the methods when adapting them for a given purpose. Of greatest importance then is the interplay between validity, precision, sensitivity, specificity and reproducibility (these terms are used in the sense defined by the above-mentioned study group (3)).

The present knowledge of this interplay seems rather scanty, and this was the reason why the present investigation was undertaken.

In this study we intended to assess the specificity and the sensitivity of recommended criteria regarding IHD and further how the acceptance of atypical symptoms as signs of IHD would affect these qualities of the diagnostic instrument

CHAPTER I

Prerequisites, material and representativity

Prerequisites

The ideal way to evaluate anamnestic symptoms and electrocardiographic changes in epidemiologic studies would be to select the series randomly from the living population. Such a sample would have to be large because of the fairly low incidence of IHD. The symptoms and signs should then be compared with the results of a method reflecting or directly demonstrating coronary and myocardial lesions. But most of the methods available for demonstrating and recording IHD *in vivo* and feasible in such a study still appear to have various inherent sources of error difficult to evaluate. This would limit the value of such an investigation. Demonstration of IHD with the accuracy desirable for the purpose in view thus still seems possible only in a post mortem series.

The most suitable method then would be a long-term prospective study of a representative group examined at relatively short intervals for symptoms and signs of IHD. After death all the relevant findings made *intra vitam* should be compared with those made *post mortem*. But objections may be raised also against such a procedure. For instance it would be difficult and probably not always possible to record the symptoms between the last follow-up and death, which may be a very informative period. Moreover such a procedure would require a high autopsy rate.

Investigations of this type are, however, in progress in various parts of the world (2, 66, 67). Since most of these investigations have not yet been concluded and since the autopsy frequency has often been low, the results hitherto obtained do not allow any generally valid conclusions.

In Malmö, a town in the south of Sweden with some 250 000 inhabitants, a prospective investigation of this type is planned as a link in a search for aetiological factors and as a basis for preventive measures. Such a combination is useful from a practical point of view. Malmö is well suited for such an investigation because it is a one-hospital town and because such a high percentage of residents of Malmö dying there are autopsied. These were the chief reasons why WHO recommended Malmö for epidemiologic investigations.

When planning this prospective study it was, however, clear that the combination of search for aetiological factors and health survey in the same

study required thorough knowledge of the specificity and sensitivity of the criteria used It was therefore decided to perform a retrospective investigation in an autopsy series from Malmö and then first assess the specificity and sensitivity of previously recommended criteria of IHD (3 4, 5) and then in an attempt to increase the sensitivity of the diagnostic instrument add the so called atypical symptoms as mentioned in the introduction

In such a retrospective investigation the autopsy frequency should be high and the examination technique and recording of the findings at autopsy should be really satisfactory especially concerning the heart and the vessels Moreover hospital records should be detailed regarding the history of the heart and technically satisfactory electrocardiograms should be available in a high percentage of the cases

To check whether these requirements could be satisfied in the planned series the hospital records of some 50 cases were selected at random from the total material The information given in the hospital records proved sufficient to allow assessment of history of the heart in some 70 % of the random sample Electrocardiography had been done in roughly equal percentage and as a rule with 12 leads including 3 standard leads (I II and III) 3 unipolar limb leads (aVR aVL and aVF) and 6 unipolar chest leads (V_1 V_2 V_3 V_4 V_5 and V_6 or V_7) This was considered sufficient to warrant the retrospective investigation

It should however, be emphasized that the anamnestic data obtained in this way are not comparable to information obtained by questionnaires regarding the possibility of standardization and inter individual comparability This point is discussed further in the section on methods

Material

The material consisted of all residents of Malmö who had died between Jan 1 1961 and March 31, 1962 But since this group included fairly few subjects below 60 years the series was supplemented by corresponding subjects below 60 years during the period April 1, 1962 to September 30 1962 The entire series thus consisted of 2 880 subjects The autopsy frequency and the number of cases in which clinical data were available are given in Tables 1 a and b, where the cases are grouped according to age and sex Of the entire series 2 023 (70 %) were autopsied the frequency being lowest in the ages above 80 years The death certificates of the 857 (30 %) subjects who had not been autopsied were studied and the causes of death noted are discussed in association with the evaluation of the representativity of the material No search was made for hospital records of these subjects Of the 584 subjects who had been autopsied but whose hospital records had not been judged 116 had died before the age of 10 years 77 had died from accidents 98 had committed suicide and the remaining 293 had died a natural death (Table II) Of these

Table I a Autopsy frequency in Malmö during study period and number of cases in which clinical data were available

Age in years	Dead No	Autopsied		Clinical data		With clinical and autopsy data	
		No	% of all in the age class	No	% of all in the age class	No	% of all in the age class
0— 9	118	116	(98 %)				
10—19	29	25	(86 %)	11	(38 %)	11	(38 %)
20—29	34	31	(91 %)	9	(26 %)	9	(26 %)
30—39	69	57	(83 %)	24	(35 %)	23	(33 %)
40—49	160	141	(88 %)	89	(56 %)	87	(54 %)
50—59	349	300	(86 %)	203	(57 %)	201	(56 %)
60—69	498	395	(79 %)	307	(62 %)	302	(61 %)
70—79	782	538	(69 %)	442	(57 %)	433	(55 %)
80—89	713	378	(53 %)	319	(45 %)	308	(43 %)
90—	118	33	(28 %)	31	(26 %)	28	(24 %)
all ages	2 880	2 023	(70 %)	1 435	(50 %)	1 402	(49 %)

subjects 92 had died at the General Hospital but their records could not be traced

The final material for the comparison between clinical data and post mortem findings consisted of 1 402 cases The sex distribution was more equal than in the total material partly because more boys than girls had died before the age of 10 years and because the group of subjects examined at the department of forensic medicine who rarely had any hospital records included more men than women (Tables I a and b II and III)

Assessment of representativity

Originally it was intended to let the test cover all deaths in the population during a certain period of time But acceptance of a case in the final material required that the hospital records were available and that autopsy

Table II Causes of death of subjects who were autopsied but on whom clinical data were not available (584 cases) 116 of the cases included in this group had died before the age of 10

	Males	Females
Not accident	254	155
Accident (not traffic)	35	7
Traffic accident	25	10
Suicide	68	30

Table III Type of autopsy in cases where the cause of death was not accidental or suicidal but where no clinical data were available (409 cases)

	Males	Females
Forensic[1]	132	75
Other (age at death>10 yr)	53	39
Other (age at death<10 yr)	69	41

[1] i e at the department of forensic medicine

Table I b Autopsy frequency and number of cases in which clinical data were available grouped according to sex

Age in years	Males					Females				
	Dead	Autopsied		Clinical data		Dead	Autopsied		Clinical data	
	No	No	% of all in the age class	No	% of all in the age class	No	No	% of all in the age class	No	% of all in the age class
0—9	73	72	(99 %)			45	44	(98 %)		
10—19	14	13	(93 %)	6	(43 %)	15	12	(80 %)	5	(33 %)
20—29	21	18	(86 %)	3	(14 %)	13	13	(100 %)	6	(46 %)
30—39	45	34	(76 %)	12	(27 %)	24	23	(96 %)	12	(50 %)
40—49	83	70	(84 %)	32	(39 %)	77	71	(92 %)	57	(74 %)
50—59	223	191	(86 %)	105	(47 %)	136	119	(87 %)	98	(72 %)
60—69	304	237	(78 %)	176	(58 %)	194	158	(81 %)	131	(68 %)
70—79	406	250	(70 %)	235	(58 %)	376	252	(67 %)	209	(56 %)
80—89	283	157	(65 %)	129	(46 %)	430	221	(51 %)	190	(44 %)
90—	39	14	(36 %)	13	(33 %)	79	19	(24 %)	18	(23 %)
all ages	1 491	1 092	(73 %)	711	(48 %)	1 389	931	(67 %)	726	(52 %)

Table II. Age, social class and civil status of those who were not autopsied and of those who were autopsied grouped according to availability of clinical records. Subjects below 10 years of age excluded

	Not autopsied		Autopsied clin. records available		Autopsied clin. records not available	
Age mean ± SD	[illegible] ± 11.8		69 ± 16.0		60 ± 17.3	
Median age	80		[illegible]1		61	
Social class I	34	(4 %)	[illegible]	([illegible] %)	29	(6 %)
II	[illegible]06	([illegible]4 %)	406	([illegible]8 %)	173	(37 %)
III	[illegible]0[illegible]	([illegible]4 %)	300	(21 %)	140	(30 %)
Unknown or uncertain	402	(4[illegible] %)	651	(45 %)	125	(2[illegible] %)
Civil status						
Unmarried	161	(19 %)	299	(1[illegible] %)	108	(23 %)
Married	[illegible]84	(33 %)	[illegible]44	([illegible] %)	221	(48 %)
Widow(er)	34[illegible]	(41 %)	406	(28 %)	88	(19 %)
Divorced	[illegible]1	(6 %)	[illegible]6	([illegible] %)	49	(10 %)
Unknown	6	—	2	—	—	—

had been done. Some cases did not fulfil these requirements and were therefore excluded. There was, however, some information available about all the excluded groups. This information could be used in the evaluation of the effect of selection.

Thus in all cases the age, sex, civil status and social class (judged according to profession), mode of death, place of death and death certificate with noted cause of death were known. In the group autopsied at the department of forensic medicine also the results of autopsy were known, and there the heart and vessels had been examined in the same way as in the rest of the material.

A further group was excluded from some parts of the final treatment of the data, namely those autopsied cases in which hospital records were available but not complete enough to give satisfactory anamnestic information on IHD.

As the purpose of the present investigation was limited and required only comparisons of the characteristics within and between certain groups of the material, in the assessment of the representativity attention should be directed above all to the effect of selection on the distribution of these characteristics between and within the groups. The results in the present study cannot be used to ascertain frequencies, ranges of variation or other parameters in the living population.

Bias due to supplementation of the series

The fact that during a certain period only subjects who had died below 60 years of age were accepted meant a

Table 1 Comparison between following groups regarding civil status distribution among consecutive age classes in per cent (absolute number in brackets) Subjects below 30 years and subjects of unknown civil status (6) excluded

1 *Not autopsied*

	Age class													
	30—39		40—49		50—59		60—69		70—79		80—89		90—	
Single	—	(4)	32 %	(6)	14 %	(7)	14 %	(14)	21 %	(52)	18 %	(60)	12 %	(10)
Married	—	(6)	53	(10)	76	(38)	64	(64)	42	(102)	17	(55)	10	(8)
Widow (er)	—		—		—		15	(15)	31	(78)	58	(193)	73	(61)
Divorced	—	(1)	16	(3)	6	(3)	7	(7)	5	(12)	6	(21)	5	(4)

2 *Autopsied and clinical records available*

	30—39		40—49		50—59		60—69		70—79		80—89		90—	
Single	21 %	(5)	11 %	(8)	13 %	(26)	13 %	(39)	14 %	(62)	15 %	(49)	22 %	(7)
Married	71	(17)	78	(69)	74	(151)	67	(204)	49	(212)	24	(78)	[illegible]	(2)
Widow (er)	—	(1)	—	(1)	6	(12)	15	(45)	32	(143)	56	(182)	71	(22)
Divorced	—	(1)	12	(11)	6	(15)	5	(15)	5	(22)	3	(11)	—	—

3 *Autopsied but clinical records unavailable*

a Natural deaths

	30—39		40—49		50—59		60—69		70—79		80—89		90—	
Single	—	(3)	25 %	(5)	17 %	(10)	13 %	(9)	20 %	(17)	16 %	(8)	—	—
Married	—	(4)	65	(13)	71	(42)	60	(41)	44	(37)	20	(10)	—	(1)
Widow (er)	—		—		—		19	(13)	27	(23)	62	(31)	—	(1)
Divorced	—	(2)	10	(2)	12	(7)	6	(4)	[illegible]	(7)	4	(2)	—	—

b Accidental deaths

	30—39		40—49		50—59		60—69		70—79		80—89		90—	
Single	—	—	—	(2)	17 %	(3)	—	(1)	—	(1)	—	(1)	—	—
Married	—	(3)	—	(7)	44	(8)	—	(9)	—	(1)	—	—	—	—
Widow (er)	—	(2)	—	—	—	—	—	(1)	—	—	—	(4)	—	—
Divorced	—	—	—	(3)	39	(7)	—	(1)	—	—	—	—	—	—

c Suicides

	30—39		40—49		50—59		60—69		70—79		80—89		90—	
Single	22 %	(4)	35 %	(7)	22 %	(6)	—	(2)	—	—	—	—	—	—
Married	61	(11)	30	(6)	63	(17)	—	(6)	—	—	—	—	—	—
Widow (er)		(1)	—	—	11	(3)	—	(1)	—	(6)	—	(2)	—	—
Divorced	—	(2)	35	(7)	—	(1)	—	(2)	—	—	—	—	—	—

Table VI Place of death of subjects not autopsied compared with place of death of subjects who were autopsied and whose clinical records were available

	Not autopsied		Autopsied	
Malmö General Hospital	24	(3 %)	1 330	(93 %)
Malmö sjukhem (geriatric department)	387	(46 %)	70	(5 %)
Malmö östra sjukhus (mental hospital)	63	(7 %)	30	(2 %)
Other hospitals	35	(4 %)	2	—
At home	225	(27 %)	3	—
Elsewhere	90	(10 %)	—	—
Unknown	26	(3 %)	—	—

certain selection. This effect can, however, be readily eliminated either by excluding such individuals or by grouping the material according to age in the analysis of those problems in which the material should be representative also regarding age or in which values corrected for age are desired.

Bias due to exclusion of subjects who were not autopsied

In the evaluation of this group attention was directed first to social factors which were compared with corresponding factors in the final series (Tables IV and V). In addition data on place and mode of death and cause of death recorded in the death certificate were analyzed (Tables VI, VII and VIII).

As expected, the average age of the subjects not autopsied was higher than that in the final material (Table IV, $t = 12.6$, $p < 0.01$). No significant difference (Table IV, $\chi^2 = 7.33$, $p > 0.05$, fig. 3) could be demonstrated between these two groups concerning the distribution of the subjects among social classes (for criteria see section on Methods). On the other hand the groups did differ in respect of civil status in that those not autopsied included more persons who had been living alone, mainly because of a higher frequency of widows and widowers. This difference may be explained by the above demonstrated difference in age between the two series so that the older a subject is the more likely he or she is to be a widower or a widow. This assumption was corroborated by comparison of age matched subjects (Table V), which showed no difference regarding civil status between the series.

As expected, the two series differed substantially regarding the place of death (Table VI). Most of those in the final series had died at Malmö General Hospital, while most of those who had not been autopsied had died at a geriatric department or at home. In both series a few had died from accidents or had committed suicide (Table VII).

An important question in the evaluation of the representativity is whether differences existed between

Table VII Cause of death of subjects not autopsied compared with cause of death of subjects who were autopsied and whose clinical records were available

	Not autopsied		Autopsied	
Not accident	821	(97 %)	1 423	(99 %)
Accident (not traffic)	17	(2 %)	6	—
Traffic accident	1	—	—	—
Suicide	[illegible]	(1 %)	3	—

the causes of death in the two series. To check this point the causes of death judged from the diagnoses in the death certificates in the two groups were compared (Table VIII). The death certificates of those who had not been autopsied had been issued by doctors who had been looking after the patient or who had been called in immediately before or after death. The fact that these doctors had issued certificates of death without requesting *post mortem* examination ought to imply that they thought the cause of death satisfactorily explained by the clinical symptoms. The death certificates in the final series were issued by the pathologists who had performed the autopsies and in these cases their definitive death certificates were used. It is clear from Table VIII that the two groups differed substantially from one another, above all regarding the frequency of malignant diseases and of pulmonary embolism as the main cause of death. Diagnoses of various types of malignant diseases were much more common in the final series, which may be explained by the fact that subjects with malignant diseases are often cared for at hospital until death, and secondly that malignant diseases may sometimes remain concealed until autopsy. It is also clear that all malignant diseases discovered at autopsy were entered in the death certificate even when such diseases were not the cause of death. Also pulmonary embolism was noted much more often in the final group (16 %) than among those who were not autopsied (less than 1 %). This difference is, of course, due to the better possibilities of detecting the condition at autopsy.

In this investigation the diagnoses of atherosclerotic disease of the heart is of most interest. In both series a diagnosis of recent myocardial infarcts was equally common. A large number of cases in both series were described as having cardiosclerosis. The import of this diagnosis made by a physician issuing a death certificate on the basis of symptomatic evidence differs from that made by a pathologist who has performed the autopsy and who has given cardiosclerosis as a contributory cause of death. Whether made *ante mortem* or *post mortem* the diagnosis is not a well defined one and its meaning probably varies from one examiner to another. Nevertheless the overall frequency of manifestations of

Table VIII Diagnoses in death certificates of those who were not autopsied compared with those who were autopsied and whose clinical records were available

	Not autopsied		Autopsied	
Total	849		1 402	
Malignant disease	93	(11 %)	591	(41 %)
Heart disease				
Recent infarction (incl thrombosis)	102	(12 %)	152	(11 %)
Old infarction	2	—	[illegible]	—
Cardiosclerosis	223	(26 %)	142	(10 %)
General atherosclerosis	43	(5 %)	232	(16 %)
Combinations of above conditions	37	(4 %)	100	(7 %)
Hypertension	45	(5 %)	115	(8 %)
Decompensation of the heart	132	(15 %)	43	(3 %)
Mors subita	9	(1 %)	1	—
Myocardosis+myocarditis	17	(2 %)	1	—
Vitium org cordis	12	(1 %)	56	(4 %)
Diseases of C N S				
Cerebral thrombosis	71	(8 %)	181	(13 %)
Hemiplegia	34	(4 %)	2	—
Cerebral arteriosclerosis	44	(5 %)	23	(2 %)
Cerebral apoplexy	10	(1 %)	—	
Cerebral haemorrhage	32	(4 %)	37	(2 %)
Combinations of above conditions	9	(1 %)	[illegible]	—
Subarachnoid haemorrhage	1	—	23	(6 %)
Senile dementia	33	(4 %)	1	—
Senility+marasmus	74	(9 %)	21	(1.5 %)
Gastro intestinal tract				
Ulcer or status after ulcer	3	—	43	(3 %)
Inflammatory conditions	2	—	33	(2 %)
Gangrene—ileus—volvulus	1	—	30	(2 %)
Peritonitis+other disease	3	—	36	(2 %)
Total		(1 %)		(10 %)
Disease of respiratory tract				
Pneumonia+bronchitis	193	(23 %)	410	(28 %)
Unspecific pulm dis +pleurisy	9	(1 %)	37	(2 %)
Pulm tb +miliary tb	2	—	22	(1.6 %)
Pulm tb cum other respir disease	—	—	19	(1.5 %)
Renal diseases				
Glomerulonephritis	—	—	10	—
Pyelonephritis	9	(1 %)	92	(6 %)
Other renal disease	19	(2 %)	39	(3 %)
Uraemia	8	(1 %)	36	(2 %)

	Not autopsied		Autopsied	
Liver disease				
Hepatitis	—	—	2	
Cirrhosis	2	—	42	(3 %)
Other	2	—	22	(1.5 %)
Gallstone	2	—	16	(1 %)
Diabetes				
Reported	45	(5 %)	49	(3 %)
With renal compl	—	—	8	—
Pulm embolism	[illegible]	—	231	(16 %)
Sepsis	10	(1 %)	54	(4 %)
Total number of diagnoses	1,339		2,970	
Number of diagnoses per individual	1.6		2.1	

atherosclerosis in the heart was roughly the same in both groups. Cardiac incompensation was noted much more often in the death certificates issued in cases not examined *post mortem*, probably because pathologists do not as a rule pay so much attention to symptoms as the clinician.

The cause of death given in the death certificates of the group not autopsied was usually one of the following diseases: pneumonia, malignant disease, diseases of the heart or diseases of the brain and cerebral vessels. The death certificates of those who had been autopsied included also diseases of the kidney, liver and gastro-intestinal tract. The death certificates of those not autopsied also naturally often included ill defined diagnoses based on symptoms, e.g. senility, cardiosclerosis, cardiac incompensation, sudden death, haemiplegia and stroke. This illustrates some difficulties when comparing and evaluating the occurrence of diseases in the two series.

As mentioned above there might be a real difference between the frequency of malignant disease in the two series. Such a difference can affect the frequencies of atherosclerotic diseases because a negative statistical correlation has been reported between cancer and atherosclerosis (10). Even if this correlation was not causal but due to the effect of mechanisms which may be taken together under the name "Berkson's fallacy" (6, 7) such factors may impair the representativity of the sample regarding the purpose of this study.

The two series also probably differ regarding type of IHD symptoms. According to information received from general practitioners in Malmö, it is the rule to refer patients with clear-cut myocardial infarction to hospital (43). This means that the atherosclerotic heart diseases noted in the death certificates of the series that had not been autopsied could have run an atypical course more often than in the series that had been examined *post*

mortem This implies a further selection because cases with typical IHD symptoms would then be overrepresented and those with an atypical clinical picture underrepresented in the final series

In summary the exclusion of the subjects not autopsied could have affected the representativity of the final series Those excluded were, on the average older and many had died at homes for the chronically sick It also seems possible that this exclusion may result in an overrepresentation of cases with typical symptoms and underrepresentation of those with atypical symptoms of IHD in the final series

Bias due to exclusion of autopsied cases in which no hospital records were available

The next group excluded from the original material consisted of cases that had been autopsied but in which hospital records were not available It is clear from Tables II and III that this group consisted largely of subjects who had been examined *post mortem* at the department of forensic medicine and had died from accidents or had committed suicide or had died at home or elsewhere outside the hospital The individuals in this group were on the average younger than in the final series (t = 10 4 p < 0 01 Table IV) This group also comprised more individuals belonging to social classes II and III than the other groups in the primary material (Table IV) partly explainable by possibilities to clarify a larger proportion in this group according to social group No attempts were made to find an explanation for this difference This group also contained a large proportion of unmarried and divorced subjects and a smaller proportion of widows and widowers (Table IV)

The lower average age was due mainly to the group that had died from accidents or committed suicide (Table V) The smaller proportion of widowers and widows was probably due to the difference in the average age of the series (Table V) *i e* as mentioned in an earlier section the older an individual the higher the probability of his or her married partner having died The higher frequency of divorced subjects can be traced to those in the 30—49 year group who had died from accidents or had committed suicide ($\chi^2 = 11\ 0$ $p < 0\ 01$ fg 1, compared with the final series)

A question that then arises is whether and in what way these excluded subjects differ from the final material in respect of ischaemic heart disease (Table IX) No great differences in *post mortem* findings were found In the males there were probably more individuals with old scars in the final series ($\chi^2 = 4\ 8$ fg 1 $p < 0\ 05$) In the females the tendency to more recent infarctions among the excluded cases was not significant ($\chi^2 = 2\ 1$ fg 1 $p > 0\ 05$) In the evaluation of the comparison it should be observed that the average age in the excluded group was lower But no correction for this bias was made because it was not of relevant interest in the present study

Table IX Comparison between following groups regarding myocardial changes demonstrated at autopsy
1 Autopsied and clinical data available   2 Autopsied but clinical data unavailable

	1		2	
	Males	Females	Males	Females
Total (those with uncertain autopsy data excluded)	690	705	292	147
No myocardial changes	401 (58 %)	507 (71 %)	180 (62 %)	96 (65 %)
Recent infarction	39 (6 %)	23 (3 %)	20 (7 %)	18 (12 %)
Myocardial scar>1 cm	134 (19 %)	80 (11 %)	37 (12 %)	17 (12 %)
Diffuse fibrosis	26 (4 %)	31 (4 %)	16 (5 %)	7 (5 %)
Scar+recent infarction	61 (9 %)	39 (5 %)	17 (6 %)	3 (2 %)
Recent infarction+diffuse fibrosis	[illegible] (1 %)	5 (1 %)	4 (1 %)	—
Scar−diffuse fibrosis	19 (3 %)	15 (2 %)	16 (5 %)	[illegible] (4 %)
Combination of recent infarction scar and diffuse fibrosis	4 —	5 (1 %)	2 —	— —
Uncertain data	(20)	(18)	(11)	(12)
Age				
arithm mean±SD	69.0±13.5	69.7±15.0	61.7±13.2	68.0±12.6
median	71	73	63	72
Total number with recent infarction	110 (16 %)	72 (10 %)	43 (15 %)	21 (14 %)
Total number with scars	218 (32 %)	138 (19 %)	72 (25 %)	26 (18 %)

In the interpretation of the findings however it is of greater interest to separate off and examine more homogeneous groups within this excluded series. The series was therefore divided into the following fairly homogeneous group: a) those autopsied at the department of forensic medicine and who had not died from an accident or committed suicide, b) those who were autopsied at the hospital but whose hospital records could not be traced, and c) those who had died from accidents or had committed suicide.

The cases of non-accidental deaths examined at the department of forensic medicine (group 2 a, Table X) includes several instances of *sudden death*, a diagnosis which according to the recommendations of the American Heart Association for example is interpreted as a sign of probable IHD. It was also found as might be assumed *a priori* that many in this group had recent infarcts: 25 % of the men and 22 % of the women (Table X). Of much greater interest however is the high frequency of infarction scars in this group: 39 % of the men and 28 % of the women had such scars (larger than 1 cm) in the myocardium. Most of these subjects had probably not been cared for at hospital because of myocardial infarction. This means that several

Table V Frequency of myocardial changes in following groups of subjects who were autopsied but on whom clinical data were unavailable
1 Autopsy at department of pathology
2 Autopsy at department of forensic medicine
a Natural cause of death (subjects below 10 years excluded)
b Accidents and suicides

	1	2 a		2 b
	Dept of path	Dept of forensic med		
		Death not accidental or suicidal		Death accidental or suicidal
		Males	Females	
Total (those with uncertain autopsy data excluded)	92	129	69	148
No changes	56 (61 %)	50 (37 %)	31 (45 %)	137 (93 %)
Recent infarction	6 (6 %)	[illegible] (14 %)	14 (20 %)	—
Myocardial scar>1 cm	10 (11 %)	25 (19 %)	13 (19 %)	6 (4 %)
Diffuse fibrosis	4 (4 5?)	9 (7 %)	5 (7 %)	5 (3 %)
Recent infarction+scar	8 (9 %)	11 (8 %)	1 (1 %)	—
Recent infarction+diffuse fibrosis	2 (2 %)	2 (1 %)		—
Scar+diffuse fibrosis	4 (4 %)	13 (10 %)	5 (7 %)	—
Recent infarction+scar+diffuse fibrosis	2 (2 %)	1 (1 %)		—
Uncertain data	—	(3)	(5)	(9)
Age				
arithm mean ± SD	68 0 ± 13 4	64 7 ± 13 2	69 0 ± 12 6	58 4 ± 17 4
median	70	65	73	60
Total number with recent infarction	18 (20 5?)	32 (25 %)	15 (22 %)	—
Total number with scars	24 (26 %)	50 (39 %)	19 (28 %)	6 (4 %)

of the cases probably ran an atypical course with the result that the exclusion of the group had a selective effect on the final material forming the basis of the present investigation

Those subjects who were autopsied at hospital but whose hospitals records could not be traced (group 1 Table V) did not differ essentially from the final series in respect of distribution of different types of IHD changes found at autopsy It would therefore appear that the effect of the exclusion of this group from the final material may be ignored in the evaluation of the results

Those who had met with fatal accidents or had committed suicide (group 2 b Table V) showed a much lower frequency of all types of myocardial changes of IHD than the other series in this material a fact which appears natural mainly because of the cause of death and partly because they were as a rule younger Six subjects *i e* 4 % of the 157 cases in this

group, had scars after myocardial infarction. No other relevant disease was found at autopsy.

It is thus clear from the results that the exclusion of those cases where the subjects were autopsied but where clinical records were not available had a selective effect on the final series. The group of interest in this respect is that of sudden deaths. In this group also previous infarctions seemed to have been rather common and probably in many cases to have run an atypical course. This accentuates the previously mentioned bias due to underrepresentation of IHD with an atypical course.

It is more difficult to evaluate the bias produced by exclusion of those who had met with a fatal accident or had committed suicide. The effect of excluding this group is more complex, and it probably influenced both the specificity and the sensitivity of the criteria studied.

Bias due to exclusion of cases with hospital records containing insufficient information on the anamnesis of IHD

As mentioned, in some cases it was difficult to judge the history of IHD because of incomplete data in the hospital records. In 316 (*22 %*) of the cases in which the hospital records were available the latter did not contain sufficient data in this respect. These subjects were autopsied and this provided a possibility of judging the selective effect of excluding those with incomplete records.

In order to evaluate this bias, the evidence of IHD found at autopsy in this group were compared with the corresponding findings in two other groups, namely one consisting of all cases with informative hospital records regarding symptoms of IHD and a subgroup within this group consisting of those who had denied symptoms of IHD. It was found that though those subjects with incomplete hospital records were, on the average older, they had fresh myocardial infarction and scars less often than those subjects whose records contained informative anamnestic data ($\chi^2 = 14.8$ fg 1, $p < 0.01$), ($\chi^2 = 4.2$ fg 1, $p < 0.05$) (Table XI). This difference could mainly be referred to more individuals having both old *and* recent infarctions in the final series ($\chi^2 = 21.5$ fg 1, $p < 0.01$).

The group with incomplete hospital records had, however, as expected more cases with both old and recent myocardial infarctions than the group that had denied symptoms of IHD (Table XI). Thus regarding both recent and old infarctions this group occupied an intermediate position between the two reference groups. The incompleteness of the notes on symptoms of IHD in these cases was probably due partly to the examiners' not having taken the histories properly and partly to the inability of the patients to give information, *e.g.* patients with cerebral vascular disease. Of the recent myocardial changes in this group most had occurred but had not been diagnosed while the patients were in hospital, which suggests that

Table VI **Comparison regarding myocardial changes of IHD demonstrated at autopsy between one group with insufficient and one group with sufficient clinical data regarding IHD anamnesis**

	Insufficient clin data regarding IHD	Sufficient clin data regarding IHD	
		Denied IHD symptoms	Total
Total (those with uncertain autopsy data excluded)	316	709	1078
No myocardial changes	215 (68 %)	593 (82 %)	692 (64 %)
Recent infarction	14 (4 %)	6 (1 %)	48 (4 %)
Myocardial scar>1 cm	55 (17 %)	64 (9 %)	159 (15 %)
Diffuse fibrosis	20 (6 %)	23 (2 %)	37 (3 %)
Recent infarction+scar	6 (2 %)	13 (3 %)	94 (9 %)
Recent infarction+diffuse fibrosis	—	2 (—)	11 (1 %)
Scar+diffuse fibrosis	5 (2 %)	[illegible] (1 %)	29 (3 %)
Recent infarction+scar+diffuse fibrosis	1 (—)	2 (—)	8 (1 %)
Age			
arithm mean±SD	74.2±11.6		67.6±15.6
median	76		69
Total number of recent infarctions	21 (7 %)	27 (3 %)	161 (15 %)
Total number of scars	67 (21 %)	84 (12 %)	290 (27 %)

many of them had run an atypical course

It has thus been shown that when some groups were excluded from the primary material which comprised all subjects in a population who had died during a certain period the exclusion had a biasing effect The average values and other characteristics can therefore probably not be regarded as representative of the primary material This bias was of relevant importance because it probably resulted in an underrepresentation of cases with atypical symptoms of IHD and of cases where the myocardial infarction passed without notable symptoms

This phenomenon has a systematic effect on the evaluation of various criteria used in the epidemiological method It leads to underestimation of false negative diagnoses with consequent overestimation of the sensitivity of the method The estimation of false positive diagnoses is however affected less because this estimation is based on comparisons of postmortem findings of IHD within groups characterized by different types of symptoms of IHD

The data presented in this chapter must be borne in mind in the evaluation of the results of the following analyses especially of those in Chapters VII and IX

CHAPTER II

Methods

Clinical data

The hospital records of the last 25 years—or more if considered necessary—of all subjects who had been autopsied and included in the primary material were analysed. This analysis was facilitated by the fact that Malmö is a one hospital town. The analysis was performed by a single person who was unaware of the *post mortem* findings. All together 3 633 hospital records (= spells in hospital) of 1 437 subjects were studied (Table VII). All the records were searched for information on IHD, electrocardiography, blood pressure and other clinical data of interest. The findings were coded according to a code devised specially for the purpose (26). As to the symptoms of IHD, the criteria regarded as typical and recommended by WHO for epidemiological use (3, 4, 5) were called typical, while IHD symptoms of other types and with other precipitating mechanisms were judged as atypical. The grounds for evaluation and exact definitions are given in detail in the chapter on the results with respect to symptoms of IHD. In the evaluation of the electrocardiograms the Minnesota code criteria were used (13). The classification of certain subgroups used in this study is given in the chapter on results.

The hospital records were analysed during a 2 year period. To reduce variation liable to occur in the judgement during such a long period, the criteria to be used were defined as precisely as possible on the basis of a preliminary study of 50 randomly selected cases (26). The coded data were transferred to IBM punch cards which were repeatedly checked for transfer errors.

Table VII. Distribution of records analysed (spells in hospital) among different departments

Medical	1 067
Surgical (incl. chest and plastic)	903
Infection (which serves also partly as dept. of med. and of respir. diseases)	412
Chronic diseases	322
Other geriatric units	97
Radiologic	166
Orthopedic	180
Gynaecologic	156
Others	330
Total	3 633

Table VIII Social class grouping. Scheme devised at the Department of Sociology, Lund 1953

	Code number		Code number
Social group I		Civil servants (of lower grade) II	18
Larger farmers	01	Elementary school teachers	[illegible]
Industrialists, wholesale merchants,		Captains (master mariners)	20
directors, managers	02	Higher domestic service	21
Executives in high position	03	Independent professions II	22
High civil servants and officers of		Other industrial occupations	
the armed forces	04	and tradesmen	23
Independent occupations I	05	Miscellaneous II	24
Houseowners I	06		
Miscellaneous I	07	*Social group III*	
		Farm foremen, etc.	30
Social group II		Crofters, etc.	31
Farm owners	10	Other farm labourers	32
Leaseholders and farmers	11	Seamen	33
Farmers' sons and sons-in-law living		Fishermen	34
at home	12	Foresters and timberfloaters	35
Artisans	13	Other workers	36
Dealers (small)	14	Civil servants of lower grade III	37
Foremen	15	Low domestic service	38
Clerks	16	Miscellaneous III	39
Shop assistants	17		

Data on the patients' age, civil status and occupation were obtained from the death certificates. The subjects were classified in occupational groups according to a scheme devised at the Department of Sociology, University of Lund (stencil 1953, Table VIII).

Information obtained retrospectively from hospital records has certain inherent sources of error. These are best seen on comparison with an imaginary situation in which the same information is obtained from questionnaires. This will show that the largest source of error in the hospital records is the lack of standardization of the history taking and entries, which may make it difficult to obtain strictly comparable data. In the present investigation this source of error was counterbalanced to some extent by the fact that the Malmö hospital is a teaching hospital and the entries are more complete and made in a more uniform way than otherwise. The majority of the patients had been examined by internists at the department of medicine or by heart specialists before an operation.

In some respect the hospital records give more exact information than questionnaires because data are available from various spells in hospital covering a long period of time. Such records give information, e.g., on the

anamnesis of the patient at the actual time of infarction. It should be emphasized that the purpose was not to make comparisons with other series but comparisons within one and the same series to get information about the validity of anamnestic data as a link in planning a prospective study.

In about 300 cases the hospital records were unsatisfactory regarding data on IHD. In roughly the same number of cases electrocardiograms were missing or not technically satisfactory. These cases formed a special group which was discussed previously in association with analysis of the representativity of the series.

Postmortem data

The autopsies were performed according to a standardised technique and the protocols were dictated during the actual examination of the organs. Several organs including the recent myocardial infarctions or suspected necroses were routinely examined microscopically. The autopsy findings were taken from the autopsy protocols, coded according to the code list and transferred to IBM punch cards.

The coronary and cerebral arteries were slit up, dissected and examined for old or recent thrombi as well as for advanced stenosis. In about half of the cases the vessels were dissected from surrounding tissue by the same prosector, the other half, by the prosectors who happened to be on duty. Advanced stenosis was said to be present if the diameter of the vascular lumen was reduced by more than 50 %. The adventitia was removed and the vessels were fixed in neutral formalin for about 24 hours and placed in plastic bags containing a small amount of neutral formalin (61, 62). The vessels were classified in a fixed state and within the bags with respect to type and extent of atherosclerosis according to the method of Sjövall and Wihman (11) with minor modifications. As the material was stored it could be judged within relatively short time in order to reduce the risk of variation of judgement. The reproducibility of the grading was checked in a small series.

Statistical methods

In this study relevant data on the clinical and autopsy findings and grading of atherosclerosis were transferred to a single punch card.

Conventional statistical methods were used. The frequency of clinical and/or autopsy data was not regularly compared with the corresponding findings in other investigations because the criteria and the methods used often differ from one series to another and thereby limit the value of such comparisons.

CHAPTER III

Anamnestic data of ischaemic heart disease and electrocardiographic recordings

The final material consisted of largely equal numbers of men and women who did not differ significantly from one another in respect of age (Fig 1) The distribution according to social class civil status and mode of death is given in the description of the analysis of the representativity of the material (Tables IV V VI VIII)

Anamnestic IHD symptoms

Definition of symptoms of IHD

As typical *angina pectoris* was accepted in accordance with recommendations by an expert group from WHO (5) effort angina as defined by Rose (63) These symptoms are defined as follows

Chest pain or discomfort with these characteristics

1 The site must include *either* the sternum (any level) *or* the left arm and left anterior chest (defined as the anterior chest wall between the levels of clavicle and lower end of sternum)
2 It must be provoked by either hurrying or walking uphill (or by walking on the level for those who never attempt more)
3 When it occurs on walking it must make the subject either stop or slacken pace unless trinitrin (nitroglycerine or a similar drug) is taken
4 It must disappear on a majority of occasions in 10 minutes or less from the time when the subject stands still

As *typical infarction* the criteria recommended by WHO for possible infarction (5 63) were used One or several attacks of severe pain across the front of the chest lasting for 30 minutes or more

The atypical symptoms may consist of deviations of all or any of the characteristics described as typical of IHD (1 2 15 17 20 21, 24 32 33) *The pain* may thus be missing entirely both in angina pectoris (sine dolore) and infarction *Pain equivalents* can arise in the form of discomfort or a feeling of compression These equivalents were included in the definition given by Rose An equivalent not included is the symptom the patient describes as shortness of breath

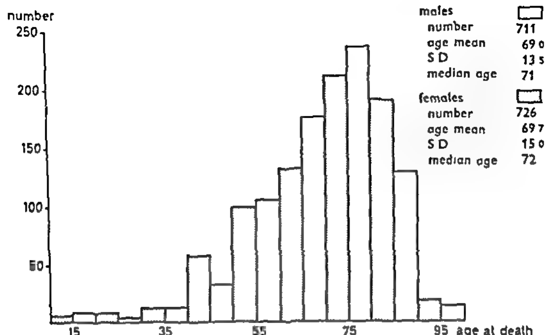


Fig. 1. Age and sex distribution of material

on exertion, a symptom which is usually judged as a respiratory symptom. But if it could be shown that this symptom is not combined with hyperpnoea but causes superficial respiration without increased respiratory frequency it can be a pain equivalent. This symptom is not simply an equivalent but also often combined with pain (44). Further, *the site* of pain may vary. It commonly occurs e.g. in the jaw or neck. The symptoms may also be referred to the right side. Scars after operations or chronic inflammatory conditions are thought to be places to which the individual often refers his coronary symptoms. This applies particularly to scars after operations on the biliary tract or stomach (1).

The *precipitating mechanism* can sometimes not be demonstrated (spontaneous angina); it may be psychic stress, postcibal, exposure to cold or recumbent position (15). Angina pectoris precipitated by a meal is judged by certain authors as a sign of advanced coronary disease (15) while others believe that it has not a particularly poor prognosis (33). Opinions differ in the same way regarding the prognostic significance of nocturnal angina. Exposure to cold may be a precipitating factor. Then local cooling such as of the face or by holding a piece of ice in one hand may trigger off angina pectoris (15, 19). This is a form which may be isolated. The type of angina which is combined with a psychic stress is not believed to have a

poor prognosis (15 33) The difficulties of drawing the line between pain suggesting angina pectoris and that suggesting myocardial infarction have also been mentioned (15 32 33)

Atypical angina pectoris was said to be present when pain or discomfort was described in the same area as in the typical cases but with the following precipitating mechanisms cold emotional exertion a meal and recumbant position Those variants with shortness of breath were also assigned to this group Pain in areas not typical of coronary pain according to Rose (63) were not accepted as atypical angina but were assigned to the group of uncertain cases

Patients with an attack of sudden pallor sweating sometimes combined with a sensation of discomfort in the chest a tendency to lie down and a tendency to faint were classified as having *atypical infarctions* No definite limits were drawn regarding the duration of the symptoms because the duration was seldom given in the records and exclusion of a case because of lack of precision in this respect would have implied the exclusion of too many cases with a history of infarction

If a subject had had typical angina pectoris he was classified as such even if he also had had co existing atypical symptoms On the other hand a subject with atypical or typical angina pectoris could be judged as having both atypical and typical infarction

Anamnestic symptoms of IHD were said to be absent only if a note had been made in the patient s record that he had denied having pain in the chest or if it was obvious that he had been doing heavily labour without symptoms referable to the heart until admission In the cases where the records contained no notes about the condition of the heart the information was said to be incomplete and as mentioned above these cases were assigned to a special group

Distribution of atypical and typical symptoms

172 (12 %) cases had typical angina pectoris Of these subjects 100 were males and 72 females The clinical picture in these cases was fairly homogenous and there was therefore no reason to subgroup In 70 (5 %) of the subjects (40 men and 30 women) the angina pectoris had been atypical The group was heterogeneous from the point of view of symptoms and subdivision for further analysis would have been of interest But the group was too small for such subdivision A third group of angina pectoris comprised cases with status anginosus defined as more or less continuous retrosternal pain for more than one day and increasing on exertion and not disappearing completely during rest or treatment with nitroglycerine Only 7 subjects 3 men and 4 women had shown such a clinical picture

The infarction episodes were grouped as mentioned into typical and atypical and into previous and recent From an epidemiological point of view previous episodes of infarction are of

Table XII Relation between different forms of angina pectoris and different types of anamnestic symptoms of myocardial infarction

	Angina pectoris				
	None	Atypical	Typical	Status anginosus	Uncertain data
Total (those with uncertain data excluded)	841	66	169	7	(331)
Anamnestic symptoms of infarction					
None	762 (91 %)	42 (64 %)	66 (39 %)	1	(15)
Atypical previous	6 (1 %)	1	2 (1 %)	—	—
Typical previous	18 (2 %)	9 (14 %)	18 (11 %)	—	—
Atypical, recent	12 (1 %)	2 (3 %)	2 (1 %)	1	(1)
Typical recent	35 (4 %)	8 (12 %)	33 (20 %)	5 (70 %)	(7)
Combinations	8 (1 %)	4 (6 %)	48 (29 %)	—	—
Uncertain data	(33)	(4)	(3)	—	(307)

greater interest because acute infarction is rarely found in such studies

Only in 9 cases (less than 1 %) could signs of previous atypical infarction be demonstrated. In 5 of the cases the subjects had attended the hospital because of their symptoms sooner or later after the episode. Eighteen had had recent symptoms referable to this type of infarction without pain. In 45 cases (3 %) the patients had had previous typical episodes of infarction and 88 (6 %) recent symptoms of typical infarction. Sixty had had both a previous and a recent infarction. In most of these with recurrent infarction (54 cases) the attacks had been typical on both occasions. Only one subject had had atypical attacks on both occasions. Five had combinations of atypical and typical symptoms of infarction.

Invariably symptoms of IHD were more common among the men than among the women. The ratio in each of the groups was about 10 : 7 (for details see Tables XXII and XXIII). It seems that this difference cannot be ascribed to a skew sex distribution or differences in ages (Fig. 1). It probably reflects a real difference between the sexes regarding the tendency to develop IHD. This difference is well known and is larger in the lower than in the higher age groups.

As known, angina pectoris is often ushered in by infarction and angina pectoris is often complicated by infarction. This was also the case in the present material (Table XIV), where 36 % with atypical angina pectoris and 61 % of the cases with typical angina were combined with an episode of infarction of some kind.

It has been claimed that the atypical course is due to certain individuals having a reduced capacity to experience pain (1, 15). If this were the case a given individual should have recurrences of the same type of infarction

and atypical angina pectoris should more often be combined with atypical infarction and the typical form of angina pectoris should not be combined with atypical infarction But no such uniform pattern could be demonstrated among those with recurrent infarction or in the relation between angina pectoris and infarction (Table XIV) This argues against this hypothetical explanation of the atypical course

Status anginosis was combined with a picture of recent infarction in 6 of the 7 cases which fits in with the opinion that it is a preinfarction state

Electrocardiographic changes

Electrocardiographic studies were included in the analysis in order to get this objective method compared with anamnestic symptoms and post mortem findings

Definition of electrocardiographic changes

In most of the cases in which electrocardiographic recordings were available 12 leads had been used namely 3 standard leads (I II and III) 3 unipolar limb leads (aVR aVL aVF) and 6 Wilson precordial leads (V_1 V_2 V_3 V_4 V_5 and V_6 or V_7) All cardiograms available in each case were checked and judged

Classification groups based on the criteria of the Minnesota code (13) were separated off This code is based on the same isolated electrocardiographic changes as are judged in clinical practice, but to enable standardisation of judgement and to obtain comparable values certain numerical values respectively quotients are given which are codified so that they can be transferred to IBM punch cards As for Q QS and T the code is constructed in such a way that changes are classified in decreasing order of severity

The electrocardiographic changes were however not coded in the different classes according to the Minnesota code Instead clusters of these criteria, defined later were used in expectance of the modifications of this code in progress (5)

From an epidemiological point of view it is of special interest to examine electrocardiographic abnormalities after previous myocardial injuries In view of the nature of the series it was therefore thought advisable to divide the series into cases with old and cases with recent electrocardiographic changes The electrocardiographic changes were classified as old if they had been demonstrated during previous spells in hospital or previous visits to the out patient department Furthermore abnormalities seen in recent electrocardiograms were classified as old if no clinical signs or symptoms of active myocardial infarction had been demonstrated at the time of the recording of the electrocardiogram The electrocardiographic changes were classified as recent if previous electrocardiograms had been normal and the change had supervened recently even in the absence of clinical signs or symptoms of myocardial infarction

Table XI Distribution of *recent* electrocardiographic changes according to sex (Figures in brackets denote corresponding number in the Minnesota code)

	Numbers and percentages	
	Males	Females
	711	724
Normal ECG	192 (27 %)	185 (26 %)
Not normal but no signs of infarction (VI 1—4 VII 2—3 VIII 0—9 IX 2—9)	102 (14 %)	69 (10 %)
S—T depression (IX 1)	152 (21 %)	204 (28 %)
Pattern of myocardial infarction (I 1—2 IX 4 V 1—2 I 3/V 3)	82 (11 %)	48 (7 %)
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	24 (3 %)	30 (4 %)
ECG missing or technically unsatisfactory	159 (22 %)	188 (26 %)

In addition electrocardiographic abnormalities were judged as recent if continuous changes had occurred during the last spell in hospital or if coexisting signs or symptoms of myocardial infarction had been demonstrated This means that the clinical picture influenced the classification of the electrocardiographic abnormalities as previous or recent

In the naming of the different groups it was presumed on the basis of clinical experience that some of the criteria in the Minnesota code were more specific for myocardial infarctions than others

The following classification was used (figures in brackets refer to corresponding groups of the Minnesota code)

Previous electrocardiographic changes

1 Normal electrocardiogram
2 Electrocardiogram not normal but no change according to 3 or 4 (group 2 thus includes *e g* AV block disturbed rhythm right bundle branch block and other small electrocardiographic abnormalities VI 1—4, VII 2—3, VIII 0—9 IX 0 2—9)
3 Infarction pattern with different localization (Q and QS and pathological R wave reduction according to I 1—2, T according to V 1—2 and V 3 if I 3 is also present)
4 Diffuse injury or injury difficult to locate (left bundle branch block, VII 1 low voltage IX 1)

Recent electrocardiographic changes

1 Normal electrocardiogram
2 Not normal but no change according to 3—5
3 Myocardial strain (ST decrease of 1 mm or more IX 1)
4 Infarction pattern with different localization (Q and QS and pathological R wave reduction I 1—2 S—T increase IX 4 T wave V 1—2 V 3 if I 3 is also present)

Table XVI Distribution of *previous* electrocardiographic changes according to sex (Figures in brackets denote corresponding number in Minnesota code)

	Numbers and percentages	
	Males	Females
	711	724
Normal ECG	301 (42 %)	301 (42 %)
Not normal but no signs of infarction (VI 1—4 VII 2—3 VIII 0—9 IX 2—9)	125 (18 %)	139 (19 %)
Pattern of myocardial infarction (I 1—2 V 1—2 I 3/V 3)	85 (12 %)	[illegible] (8 %)
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	48 (7 %)	43 (6 %)
ECG missing or technically unsatisfactory	152 (21 %)	181 (25 %)

5 Diffuse injury or injury difficult to locate (left bundle branch block VII 1 low voltage IX 1)

The third category of column I respectively V of the Minnesota code *i e* the least specific Q QS and T pattern was thus included only when they occurred simultaneously S—T decrease in association with previous electrocardiographic abnormalities was not codified because no valid information was available about simultaneous treatment if any with digitalis

In contrast to what is usual when employing the Minnesota code several cases were judged on the basis of consecutive electrocardiographic sequences taken during the same spell in hospital Therefore in some cases the evaluation was firmer than would have been possible by an isolated electrocardiogram

Tables XV and XVI give the frequencies of the different electrocardiographic changes distributed according to sex These frequencies are only of value for describing properties in the present series

Relationship between anamnestic symptoms of IHD and electrocardiographic changes

Both the anamnesis and electrocardiography have inherent sources of error as diagnostic instrument Various pathological conditions in the chest and in the upper abdomen can anamnestically simulate coronary heart disease and some diseases of the lung and brain can produce electrocardiographic abnormalities resembling those of myocardial injury (18) One might therefore *a priori* expect a certain discrepancy between the results obtained by the two methods

The analysis was made in the following way It was first judged to what extent the electrocardiogram agreed with what could be expected from the symptoms of IHD It was

also judged whether any discrepancy existed in this respect between cases with typical respectively atypical symptoms and whether any differences in the electrocardiographic pattern existed between cases with anamnestic symptoms of angina pectoris respectively of myocardial infarction. The following presentation is based on the results summarized in Tables XVII a—c.

In the group who had denied anamnestic symptoms of IHD 15 % had conduction disorders and arrhythmias and 33 % had S—T depressions 1 mm or more. In view of the composition of the series these figures were to be expected. Most of these changes were probably due to causes other than IHD and then mainly to the effect of digitalis and electrolyte changes. Thus 108 subjects in the final series had taken digitalis occasionally or continually during their last spell in hospital. It was however not thought desirable to exclude those cases in which digitalis had been given, but the effect of such treatment was judged separately in each case where it might have influenced the results. In the group who had denied anamnestic symptoms of IHD 2 % had electrocardiographic signs of recent myocardial infarction and 3 % had recently acquired left bundle branch block. Previous electrocardiographic changes were more common in this group. Thus 13 % had previous changes including a pattern of infarction, left sided bundle branch block or diffuse myocardial injury.

Among the *cases with angina pectoris* one might expect changes of infarction to be rather common because this condition is often combined with episodes of infarction (Table XIV). To facilitate the analysis of the cases with angina pectoris, those with and without infarction episodes respectively were analysed separately. Of the group with angina pectoris without an infarction episode, about 30 % had signs of previous pattern of infarction whether the angina pectoris had been typical or atypical. Among the cases with typical angina pectoris there were many more in which the recent electrocardiogram showed S—T depression (1 mm or more) (61 %) than among those with atypical angina pectoris (39 %, $\chi^2 = 5.6$, fg. 1, $p < 0.05$) or without IHD anamnesis (33 %, $\chi^2 = 19.9$, fg. 1, $p < 0.01$). This difference could perhaps be due to the fact that there were more patients with typical angina pectoris who had been treated with digitalis than among those with atypical angina pectoris respectively among those without a history of IHD. This hypothesis is supported by the fact that the difference found between the two types of angina pectoris disappeared on elimination of the influence of the cases that had received digitalis ($\chi^2 = 2.3$, fg. 1, $p > 0.05$). On the other hand the significant difference persisted between cases with typical angina pectoris and cases without anamnestic symptoms of IHD even after elimination of the influence of those treated with digitalis ($\chi^2 = 12.3$, fg. 1, $p < 0.01$). This suggests that also other factors may be of significance and in the

Table XVII ECG in different types of anamnestic symptoms of IHD Number in brackets denote numbers in Minnesota code Expected ECG changes are enframed Cases with ECG missing or technically unsatisfactory are not included in totals or percentages

a No anamnestic symptoms of IHD and atypical angina pectoris

	No IHD in anamnesis	Atypical angina pectoris			
		With previous infarction in anamnesis	With recent infarction in anamnesis	With previous and recent infarction in anamnesis	Without infarction in anamnesis
Total	571	10	10	4	36
Recent ECG changes					
Normal ECG	[270 (47 %)]	3	—	—	11 (30 %)
Not normal ECG but no signs of infarction (VI 1—4 VII 2—3 VIII 0—9 IX 2—9)	90 (15 %)	2	—	1	6 (17 %)
S—T depression (IV 1)	187 (33 %)	4	1	1	[14 (39 %)]
Infarction pattern (I 1—2 IV 4 V 1—2 I 3/V 3)	12 (2 %)	1	[8]	[2]	5 (14 %)
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	16 (3 %)	—	[1]	[ ]	—
ECG missing or technically unsatisfactory	(101)	—	—		(7)
Previous ECG changes	581	10	8	4	39
Normal ECG	388 (66 %)	3	[illegible]	—	22 (56 %)
Not normal ECG but no signs of infarction (VI 1—4 VII 2—3 VIII 0—9 IX 2—9)	130 (22 %)	2	1	—	7 (18 %)
Infarction pattern (I 1—2 V 1—2 I 3/V 3)	3[illegible] (6 %)	[4]	1	[4]	[5 (13 %)]
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	33 (6 %)	[1]	3	[—]	[5 (13 %)]
ECG missing or technically unsatisfactory	(178)	—	(2)	—	(0)

Table XVII b Typical angina pectoris

	Typical angina pectoris			
	With previous infarction in anamnesis	With recent infarction in anamnesis	With previous and recent infarction in anamnesis	Without infarction in anamnesis
Total	20	31	42	62
Recent ECG changes				
Normal ECG	7 (35 %)	1 (3 %)	3 (7 %)	8 (13 %)
Not normal ECG but no signs of infarction (VI 1—4 VII 2—3 VIII 0—9 IX 2—9)	2 (10 %)	3 (10 %)	8 (19 %)	13 (21 %)
S—T depression (IX 4)	8 (40 %)	1 (3 %)	4 (9 %)	38 (61 %)
Infarction pattern (I 1—2 IV 4 V 1—2 I 3/X 3)	—	24 (77 %)	19 (45 %)	1 (2 %)
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	3 (15 %)	2 (6 %)	8 (19 %)	2 (3 %)
ECG missing or technically unsatisfactory	—	(4)	(0)	(4)
Previous ECG changes	20	29	47	65
Normal ECG	4 (20 %)	16 (55 %)	3 (6 %)	27 (42 %)
Not normal ECG but no signs of infarction (VI 1—4 VII 2—3 VIII 0—9 IX 2—9)	2 (10 %)	7 (24 %)	2 (4 %)	18 (28 %)
Infarction pattern (I 1—2 V 1—2 I 3/X 3)	10 (50 %)	5 (17 %)	34 (72 %)	12 (18 %)
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	4 (20 %)	1 (3 %)	8 (17 %)	8 (12 %)
ECG missing or technically unsatisfactory	—	(6)	—	(1)

Table VIII c With anamnestic symptoms of infarction without angina pectoris

	Atypical		Typical		
	Previous	Recent	Previous	Recent	Combinations
Total	6	11	18	33	8
Recent ECG changes					
Normal ECG	1	2	5 (28 %)	2 (6 %)	—
Not normal ECG but no signs of infarction (VI 1—4 VII 2—3 VIII 0—0 IX 2—9)	3	1	6 (33 %)	2 (6 %)	—
S—T depression (IV 1)	1	—	7 (39 %)	3 (10 %)	1
Infarction pattern (I 1—2 IV 4 V 1—2 I 3/V 3)	1	6	—	24 (74 %)	4
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	—	2	—	2 (6 %)	3
ECG missing or technically unsatisfactory	—	(1)	—	(2)	—
Previous ECG changes	6	11	[illegible]	27	8
Normal ECG	1	7	5 (27 %)	18 (67 %)	2
Not normal ECG but no signs of infarction (VI 1—4 VII 2—3 VIII 0—9 IX 2—9)	2	[illegible]	5 (27 %)	5 (19 %)	1
Infarction pattern (I 1—2 V 1—2 I 3/V 3)	3 (50 %)	1	6 (33 %)	2 (7 %)	3
Left bundle branch block or diffuse myocardial damage (VII 1 IX 1)	—	—	2 (10 %)	2 (7 %)	2
ECG missing or technically unsatisfactory	—	*(1)*	—	(8)	—

light of the characteristics in the respective groups it seems reasonable to assume that the cause was partly the occurrence of real IHD

In those cases where the history of angina pectoris had been complicated by *previous* episodes of infarction there were, as expected, more often (50—70 %) signs of previous infarction or left bundle branch block in the electrocardiogram without any certain difference between different forms of angina pectoris In the group with angina pectoris and *recent* infarction episode expected electrocardiographic changes were common (83—90 %) without any significant difference in frequency between typical and atypical angina pectoris Only rarely did those with angina and recent infarction episode have a normal electrocardiogram In those cases where angina pectoris was combined with repeated episodes of infarction the expected electrocardiographic changes were often found without any certain difference between the two types of angina pectoris

The cases with *isolated previous episodes of infarction* showed the expected electrocardiographic changes in about 50 % without any difference between typical and atypical infarction Neither was any difference found in this respect between subjects with *recent infarction* (79 and 72 %) Subjects with *recurrent infarction* without angina pectoris had largely the expected electrocardiographic changes

Comments

This analysis showed a large discrepancy between the anamnesis of IHD and electrocardiographic changes in the form of arrhythmias and S—T depressions It appears probable, as will later be apparent that these changes were fairly non specific as far as IHD is concerned This raises the question to what extent the results are conclusive for population studies In the present series there were a number of sources of error e g effect of medicine and electrolyte disturbances, while the frequency of such sources of error would probably be small in a population study with representative material in respect of age This means that S—T depressions and to a certain extent arrhythmias and conduction disorders may be much more specific of IHD in such an investigation If the population study is concerned mainly with the higher age groups, however these sources of error may increase substantially, especially in areas where digitalis and diuretics are used for wide indications This should be considered both in the planning of population studies and in the interpretation of the results

In the present study cases with discrepancies between the anamnesis of IHD and expected electrocardiographic recordings are of greater interest than those in which there was no such discrepancy In this chapter we have reported discrepancies cases who had denied anamnestic symptoms of IHD had electrocardiographic changes indicative of IHD in 15 % and

cases with a history of a previous infarction episode or angina pectoris had normal electrocardiograms in about 25 %. The importance of these discrepancies will be studied in a later chapter where a comparison is made between postmortem findings in the different groups characterized by discrepancies between their anamnesis and electrocardiographic pattern.

Of special interest for one of the purposes of this study is that no sure difference could be demonstrated in the comparison of electrocardiographic changes between cases with typical and atypical symptoms.

CHAPTER IX

Ischaemic heart disease demonstrated post mortem

IHD according to definition is a condition with decreased transport of blood to the myocardium implying an insufficient supply of oxygen to the muscle of the heart. The condition is believed to be due mainly to vascular changes of atherosclerotic nature which *per se* or by favouring thrombotic complications causes obstruction or occlusion of one or more coronary arteries. Obstruction is believed to cause a temporary ischaemic condition usually without accompanying myocardial necrosis and this condition produces symptoms largely in the form of angina pectoris. Occlusion is believed to result in myocardial necrosis and then the clinical picture of myocardial infarction. Opinions differ on various stages in this process. The role played by atherosclerosis has been questioned (10, 39). Thrombosis has been regarded as secondary to myocardial infarction (38), and the role played by hypoxaemia respectively necrosis in the causation of clinical symptoms has been discussed (1, 14, 15).

To enable a differentiated analysis the changes in the coronary arteries and in the myocardium were studied in the present investigation. Only the severe changes of the coronary arteries were included because the purpose was mainly to reveal associations between the clinical findings and morphological signs of IHD. The term 'no special vascular changes' is therefore to be understood as the absence of severe stenosis or thrombosis and most of these cases had atherosclerotic changes of the coronary arteries, sometimes widespread.

Relation between lesions of the coronary arteries and of the myocardium

The types and frequencies of coronary and myocardial changes are given in Tables XVIII a and b. Both types of changes were more common in the men than in the women. This fits in with the results previously presented regarding anamnestic and electrocardiographic evidence of IHD. The figures agree well with those found in previous investigations in Malmö (49, 64). The results presented in these tables give information only on the present series and will not be discussed further.

Table XVIII a Severe coronary lesions demonstrated at autopsy in males and females

	Males 711		Females 726	
No arterial changes of this type	486	68 %	577	79 %
Fresh thrombosis	44	6 %	20	3 %
Old thrombosis	17	2 %	10	1.5 %
Severe stenosis	78	11 %	63	9 %
Old and fresh thrombosis	10	1.5 %	5	1 %
Stenosis and fresh thrombosis	22	3 %	14	2 %
Stenosis and old thrombosis	20	3 %	6	1 %
Stenosis+old+fresh thrombosis	5	1 %	1	—
Uncertain postmortem data	29	4 %	30	4 %

As expected a covariation was found between the coronary and myocardial lesions The covariation was however not complete Advanced vascular changes were most often associated with myocardial lesions while advanced myocardial lesions were often seen without associated severe vascular changes The findings are given in detail in Table XIX and Fig 2

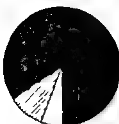
For simplicity Fig 2 is semi schematic Thus cases with myocardial infarction associated with advanced diffuse myocardial fibrosis were assigned to the respective groups of infarction In groups with only one case with fibrosis the case was not included Those cases in which no post mortem data were available or in which the results of autopsy were not clearly defined were excluded before calculation of the frequencies

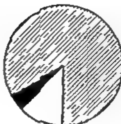
Appearances of the myocardium in different coronary lesions In 13 % of

Table XVIII b Myocardial lesions demonstrated at autopsy in males and females

	Males 711		Females 726	
No myocardial changes	401	56 %	507	70 %
Recent infarction	39	5 %	23	3 %
Myocardial scar>1 cm	134	19 %	80	11 %
Diffuse fibrosis	26	4 %	31	4 %
Recent and old infarction	61	8.5 %	39	5 %
Recent infarction and diffuse fibrosis	6	1 %	5	1 %
Old infarction and diffuse fibrosis	10	3 %	15	2 %
Recent infarction+old infarction+diffuse fibrosis	4	0.5 %	5	1 %
Uncertain postmortem data	21	3 %	21	3 %
Total with recent infarction[1]	110	16 %	72	10 %
Total with old infarction[1]	218	32 %	139	12 %

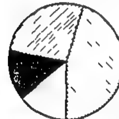
[1] Percentage calculated after exclusion of cases with uncertain postmortem data

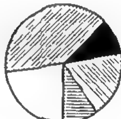


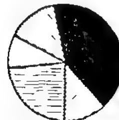












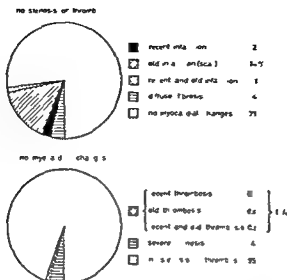


Fig 7 Relation between coronary arterial stenosis or thrombosis and myocardial changes

the cases with old thrombosis or combined old and recent thrombosis no myocardial changes could be demonstrated In 6 % was the myocardium of normal appearance in the presence of recent thrombosis No myocardial lesions were found in 23 % of the cases with severe stenosis

Coronary lesions in the presence of myocardial infarction In 60 % of all cases with myocardial scars no particular vascular lesion was demonstrated This figure is somewhat higher than that reported in other series (1 12 17 46) This may however be due to the selection of such series which usually consist of cases with clinically diagnosed myocardial infarction Among the cases with myocardial scars the commonest arterial lesion was severe stenosis (23 %) while old occluding thrombi were found in only 13 % In subjects with recent myocardial infarction the vascular changes were more common namely 56 % with recent occluding thrombi which in some cases were combined with old thrombosis or severe stenosis It is remarkable that in 18 % of the cases with recent infarction only severe stenosis was demonstrated In 22 % no relevant coronary arterial changes were seen a frequency agreeing largely with those found in previous investigations (12 17 46) Among the cases with both recent infarction and myocardial scars severe stenosis without other vascular changes was found in 24 % and no arterial changes in 9 %

Comments

The fact that occlusion of a coronary artery need not produce myocardial

Table XIX Comparison between coronary arterial and myocardial changes demonstrated at autopsy (see fig 2)

	Total	No spec arterial changes	Fresh thromb	Old thromb	Severe stenosis	Fresh + old thromb	Fresh thromb + severe stenosis	Old thromb + severe stenosis	Fresh + old thromb + severe stenosis	Uncertain post mortem data
Total		1 003	64	27	111	16	36	20	6	57
No myocardial changes	908	815	4	3	33	2	1	3	—	17
Recent myocard infarction	62	15	29	1	7	3	7	4	—	—
Scar > 1 cm	214	130	4	15	16	4	6	5	1	3
Advanced diffuse fibrosis	27	10	1	1	14	—	—	—	—	1
Recent infarction + scar	104	10	23	4	25	7	22	12	5	1
Recent infarction + diffuse fibrosis	11	2	3	—	5	—	—	—	—	1
Scar + diffuse fibrosis	34	15	—	3	11	—	—	5	—	—
Uncertain post mortem data	40	6	—	—	—	—	—	—	—	34

infarction has been explained by the assumption that the occlusion has developed gradually and that collaterals have had time to develop (12 50) This mechanism can partly explain the cases with recent thrombosis and coexisting stenosis without consequent myocardial infarction In cases without stenosis occlusion without consequent myocardial necrosis may perhaps be due to a good collateral circulation as an anatomical variant it having been shown that several cases without signs of IHD *post mortem* sometimes have well functioning collaterals (20, 43) It should also be borne in mind that myocardial lesions possible to detect with routine methods do not occur until several hours after the acute occlusion

The pathogenesis of severe coronary vascular stenosis has been widely discussed In most cases it is probably a purely atherosclerotic narrowing but the hypothesis has been suggested that the stenosis is usually due to mural thrombi initially occluding or obstructing the lumen which are afterwards organised and lined with intima (40) Careful microscopical studies appear to support this assumption (39, 20) This mechanism would perhaps explain why scars after infarcts are associated with only severe arterial stenosis in so many cases (23 % in this study) and also explain the pathogenesis of the myocardial infarction in these cases But the fairly large number of recent infarcts with only severe arterial stenosis weighs against the general validity of this hypothesis

The incorporation of a thrombus in the vascular wall may perhaps also explain why scars after infarcts are so often seen in the absence of severe coronary arterial lesions (60 % in this study) The occluding thrombus may have retracted or recanalized and become lined with intima to such an extent that even severe stenosis no longer persists Another explanation is that in spite of the careful examination of the arteries made in this study old thrombi may have been missed Some authors suggest the possibility of transient coronary arterial spasm as a cause of myocardial infarction but this possibility is not widely accepted (for survey see 38) Another possibility is that some infarcts are not of atherosclerotic origin (20 48)

The poor agreement between myocardial infarcts and coronary vascular lesions should be borne in mind in the interpretation of coronary angiograms especially in patients with symptoms of IHD without definite angiographic signs of stenosis

CHAPTER X

Relation between anamnestic symptoms and post mortem findings

This chapter concerns the relation between anamnestic symptoms of IHD and the morphological changes demonstrated post mortem in the coronary arteries and myocardium. The significance of coronary arterial lesions without associated myocardial lesions will also be discussed.

In the analysis the series was divided into groups with various combinations of typical and atypical symptoms (Table XX). The frequency distribution of various myocardial changes was studied in the respective groups. In the further analysis each group was subdivided into cases with and without myocardial lesions after which the number with various arterial changes was analysed (Tables XX a, b and c).

Coronary and myocardial lesions in the absence of anamnestic symptoms of IHD

In the evaluation of the epidemiological method the number of false negative diagnoses is of importance, i.e. those who were erroneously judged as not having the disease. It was found that all together 14 % (109 cases) of the cases without anamnestic symptoms of IHD had myocardial infarction. The majority (82 cases, 11 %) of these had only scars after infarcts, 10 (1 %) had only recent infarcts while 17 (2 %) had both recent and old myocardial infarcts.

Of the cases without symptoms of IHD and without myocardial lesions 23 (4 %) had severe coronary stenosis. It can however be questioned whether such stenosis had any haemodynamic effect of clinical significance. Further two cases had both recent and old thrombi and one an old thrombus and severe stenosis. These cases with thrombosis but without anamnestic symptoms and without myocardial lesions are of interest in the discussion of the importance of the collateral circulation mentioned in a previous section. But the combination in question was seen in only 3 cases, which limits the value of the finding.

Subjects who had myocardial infarction at autopsy and who had denied IHD symptoms are as mentioned of interest in the evaluation of an epidemiological method because they impair the sensitivity of the anam

Table XX My car l al an l c ronary arter al l n ges den le l at a t j sy l gro ps l l l ffere t types of IHD sy plo s
Cases l a a of sy da n are excl le l fro tl e totals an l perce ges
n No IHD a a es s an l typical a gi n pector s respect ely

	No IHD n esis	Typical angina pectoris			
		With pre s f r th ln n a es s	With re nt farcti n sis	With pre s lr t i farctio n esis	With t i f rct on i n nes s
My r l l cl ges	711	19	31	11	6
N yocar l l anges	610(8? %)	5(?5 %)	—	—	?7(11 %)
R t nfar t	8 (1 o)		1 (3 o)	1	—
Scar>1 c	73(10 %)	9(1? %)	—	1 (7 o)	= (38 o)
D ff e f l sls	? (3)	1 (%)	—	—	(8)
S r l r ce t infar lo	1 (?)	?(10 o)	13(6)	(0 o)	3 (5 o)
Re e t farcti an l lff e f l ro s		—	—	1 (9 %)	—
Scar+ ff e f l ros s	9 (1 o)	3(16 %)	1 (1)	3 (7 o)	5 (8)
R t f r tl +sc r+ Iiff e f l ro s	=	—	(6 o)	(1 %)	—
U ert n j ost tem lata	(18)	(1)	(1)	(3)	(1)

	Without coronary changes	Total	With coronary changes	Total	With coronary changes	Total	Without coronary changes	Total	With coronary changes	Total
Coronary arterial changes	603	711		19		31		11		6
N l ges	577(95)	673(91 %)	r	11(8 %)	—	(6 %)	—	(11 %)	3	40(61 %)
Fresl tl l ls	—	(31 %)	—	—	—	1?(35 %)	—	5(11 %)	—	(3 o)
Ol l tl ro l o ls	—	1 (%)	—	?(10 o)	—	1 (3 %)	—	3 (6 %)	—	1 (1 %)
S ere al ls	?3 (1 %)	11 (6 %)	—	3(16)	—	9(6 %)	—	1 (31 %)	3	18(?8 o)
Ir l a l tl tlr losls	?	3	—	—	—	? (6 %)	—	1 (9 %)	—	—
Ir sl tl ro nl osis n l e ere stenosis	—	1	—	1 (%)	—	(1)	—	6(14 %)	1	(3 o)
Ol l tl ro l o ls an l se ere osis	1	3	—	?(10 %)	—	? (6 %)	—	1 (9 %)	—	? (3 %)
Ol l a l fresl tl o losis+se ere tenosis	—	—	—	—	—	1 (3 %)	—	? (1 %)	—	—
Unce tal j o orte lata	(7)	(21)	—	(1)	—	(1)	—	(3)	—	(1)

Table XX b. Atypical angina pectoris

	Atypical angina pectoris			
	With previous infarction in anamnesis	With recent infarction in anamnesis	With previous and recent infarction in anamnesis	Without infarction in anamnesis
Myocardial changes	9	10	3	43
No myocardial changes	5	1	—	15 (35 %)
Recent infarction	—	2	—	2 (5 %)
Scar>1 cm	2	1	1	18 (42 %)
Diffuse fibrosis	—	—	—	3 (7 %)
Scar and recent infarction	1	3	2	2 (5 %)
Recent infarction and diffuse fibrosis	—	1	—	—
Scar+diffuse fibrosis	1	1	—	3 (7 %)
Recent infarction+scar+diffuse fibrosis	—	1	—	—
Uncertain postmortem data	(1)	—	(1)	(2)

	Without myocard changes	Total	Without myocard changes	Total	Without myocard changes	Total	Without myocard changes	Total
Coronary arterial changes		9		10		3		43
No changes	5	7	1	3	—	—	15	29 (67 %)
Fresh thrombosis	—	1	—	2	—	1	—	5 (12 %)
Old thrombosis	—	1	—	—	—	—	—	1
Severe stenosis	—	—	—	3	—	1	—	4 (9 %)
Fresh and old thrombosis	—	—	—	1	—	—	—	1
Fresh thrombosis and severe stenosis	—	—	—	1	—	—	—	—
Old thrombosis and severe stenosis	—	—	—	—	—	1	—	3 (7 %)
Old and fresh thrombosis+severe stenosis	—	—	—	—	—	—	—	—
Uncertain postmortem data	—	(1)	—	—	—	(1)	—	(3)

Table XV c With infarction in anamnesis without angina pectoris

	Atypical infarction in anamnesis		Typical infarction in anamnesis		
	Previous infarction in anamnesis	Recent infarction in anamnesis	Previous infarction in anamnesis	Recent infarction in anamnesis	Previous and recent infarction in anamnesis
Myocardial changes	6	12	17	32	6
No myocard. changes	1	4	7 (41 %)	6 (19 %)	—
Recent infarction	—	4	2 (12 %)	11 (34 %)	—
Scar>1 cm	[illegible]	2	5 (29 %)	5 (16 %)	1
Diffuse fibrosis	—	—	1 (6 %)	1	—
Scar and recent infarction	1	—	1 (6 %)	7 (22 %)	4
Recent infarction and diffuse fibrosis	—	1	—	2 (6 %)	—
Scar+diffuse fibrosis	1	1	1 (6 %)	—	—
Recent infarction+scar+diffuse fibrosis	—	—	—	—	1
Uncertain postmortem data	—	—	(1)	(3)	(2)

	Without myocard changes	Total	Without myocard changes	Total	Without myocard changes	Total	Without myocard changes	Total	Without myocard changes	Total
Coronary arterial changes		6		11		17		32		6
No changes	1	3	4	6	6	12 (70 %)	3	6 (19 %)	—	—
Fresh thrombosis	—	—	—	—	—	2 (12 %)	2	15 (47 %)	—	—
Old thrombosis	—	—	—	—	—	1 (6 %)	—	—	—	—
Severe stenosis	—	1	—	4	1	2 (12 %)	1	5 (16 %)	—	3
Fresh and old thrombosis	—	1	—	1	—	—	—	—	—	—
Fresh thrombosis and severe stenosis	—	—	—	—	—	—	—	4 (12 %)	—	1
Old thrombosis and severe stenosis	—	—	—	—	—	—	—	1	—	[illegible]
Old and fresh thrombosis+severe stenosis	—	1	—	—	—	—	—	1	—	—
Uncertain postmortem data	—	—	—	(1)	—	(1)	—	(3)	—	(2)

nestic method This group will be analysed separately in a later section (page 67)

Coronary and myocardial lesions in angina pectoris

Of the patients with angina pectoris combined with a history of infarction the course of the infarction was atypical in only a few. Neither did atypical angina pectoris prove to be more frequently combined with an atypical course of the infarction. Therefore all cases with a previous history of infarction irrespective whether they had run a typical or atypical course were pooled. This was also done with those with recent episodes of infarction.

Of the subjects with typical angina pectoris with previous and/or recent myocardial infarction the frequency of expected myocardial changes was about 70 %.

Of individuals with typical angina pectoris without anamnestic episodes of infarction there was myocardial scars in about 50 %. In 27 cases (41 %) there were no myocardial lesions. Among those several cases of severe arterial stenosis may be expected but only 3 had severe stenosis. Thus severe stenosis not associated with myocardial infarction seemed not to be a common cause of angina pectoris in this series. On the other hand myocardial scars were found in 51 % of the cases with typical angina pectoris without anamnestic symptoms of myocardial infarction.

The results obtained in the group with atypical angina pectoris were largely the same as those found in subjects with typical angina. Of those with atypical angina and with previous symptoms of infarction myocardial and coronary vascular changes were somewhat less common (55 %), but the difference was not statistically significant ($\chi^2 = 0.92$ fg 1, $p > 0.05$). Since the various subgroups were fairly small further analysis was not possible. It was, however of interest to note that of the subjects with atypical angina without anamnestic symptoms of myocardial infarction and which had no myocardial lesions post mortem (15 cases), none had severe coronary stenosis.

Comments

Of the entire group with typical or atypical angina pectoris 53 showed no myocardial lesions. Of these only 3 had coronary arterial stenosis. Thus, in 50 of the cases with anamnestic symptoms suggestive of IHD in the form of angina no coronary or myocardial changes had been seen. This suggests that the symptoms might be due to factors other than IHD. These cases are of interest in the evaluation of the epidemiological method because they represent a systematic source of error which reduces the specificity of the anamnestic criteria. To form an opinion of the background of these cases they were analysed separately and are discussed in a later section (page 78).

The results may also be used as a

basis for the discussion of the significance of coronary arterial stenosis as the cause of the symptom angina pectoris. As previously mentioned only advanced stenosis was considered since most subjects above 50 years have atherosclerotic changes of the coronary arteries. Thus classification of cases into two groups with and without atherosclerosis is of no interest in the evaluation of presumed clinical sequelae. It was shown previously (Table XIX, Fig. 2) that coronary artery thrombosis and/or myocardial lesions are more common among cases with advanced coronary stenosis. This result is in agreement with the concensus of opinion that stenosis predisposes to myocardial infarction. But the significance of coronary stenosis in the causation of the symptom of angina pectoris in subjects without myocardial infarction is not quite clear (14). As previously mentioned coronary arterial stenosis was found in 6 % of the subjects with angina pectoris without myocardial infarction. This is roughly the same percentage as in the group who had denied anamnestic symptoms of IHD and it did not differ significantly from the frequency in the group with neither anamnestic symptoms of IHD nor myocardial lesions ($\chi^2 = 0.42$, fg. 1, $p > 0.05$). This means that in this series judged in retrospect no definite association was found between angina pectoris and the occurrence of coronary arterial stenosis without myocardial infarction. In previous investigations of the relations between clinical symptoms and the post mortem findings also a high frequency of scars has been found in the series with angina pectoris (14, 50, 65), but the significance of coronary arterial stenosis in the absence of myocardial infarction has however not been made the subject of a systematic study.

A question that then arises is whether subjects with angina pectoris *and myocardial scars* have arterial stenosis more often than subjects with anamnestic symptoms of infarction and myocardial scars. This proved to be the case (Table XXI). It was also of interest to note that concerning the occurrence of arterial stenosis also cases with atypical angina pectoris more closely resembled those with only anamnestic symptoms of infarction or "silent" infarctions.

The typical picture af angina pectoris is thus more often associated with arterial stenosis than other anamnestic symptoms of IHD, a finding in accord with the generally accepted conception. But the arterial stenosis in these cases was associated with myocardial scars even when the subject's history contained no notes of any infarction.

This prompts the question: Why have most of these cases with angina pectoris and coronary stenosis also myocardial scars though they have no anamnestic symptoms of infarction. Two possibilities may be discussed. It has been suggested that angina pectoris is always ushered in by myocardial infarction (17, 51) and this would agree with Duguid's opinion that severe vascular stenosis is

Table XXI 1 Distribution of coronary stenosis among cases with different IHD anamnesis. Only cases with myocardial scar are included

	1 No IHD in anamnesis	2 Atypical angina + infarction in anamnesis	3 Atypical angina without infarction in anamnesis	4 Typical angina + infarction in anamnesis	5 Typical angina without infarction in anamnesis	6 Infarction in anamnesis without angina pectoris
a With coronary						
stenosis without thrombosis	21 (17 %)	4 (2[illegible] %)	4 (16 %)	27 (29 %)	18 (47 %)	13 (21 %)
Others	103	13	21	70	20	50
b With coronary						
stenosis + stenosis and thrombosis	27 (22 %)	6 (38 %)	7 (28 %)	[illegible]0 (52 %)	21 (55 %)	23 (30 %)
Without stenosis	97	10	[illegible]	47	17	10

II Matrices showing χ^2 values on mutually comparison between the various groups of symptoms with regard to presence of coronary stenosis. The figures refer to symptom groups according to I. Letters denote vascular groups under I

	1	[illegible]	[illegible]	4	5	6	a	Intervals of significance	
1	—	0.8	0.0	3.8	14.9	1.0		$\chi^2 = 3.84$	p = 0.05
2	1.0	—	0.5	0.1	2.3	0.1		$\chi^2 = 6.63$	p = 0.01
3	0.5	0.1	—	1.5	6.5	0.2		$\chi^2 = 10.8$	p = 0.001
4	0.1	1.1	4.4	—	4.7	1.0			
5	15.6	1.4	4.5	0.2	—	8.0			
6	4.6	0.0	0.6	3.5	3.4	—			
	b								

not generally of atherosclerotic origin but most often a sequela after thrombosis (40) It might thus be imagined that coronary pain is due to a myocardial scar It is however, difficult to find evidence for this hypothesis in the present conception of the origin of the pain in IHD (for survey see 15 28) In the light of the electrocardiographic recordings in attacks of angina pectoris it has been suggested that every attack of pain in angina pectoris is due to necrosis of a small area of the heart muscle (14) The accumulation of several small necrotic foci may cause myocardial scarring in all cases of real angina pectoris This mechanism would also be able to explain why the repeated small attacks of pain can cease in a patient with angina pectoris after an attack of myocardial infarction It might perhaps be due to the stenosed artery becoming occluded with consequent necrosis of its entire area of supply This might also to a certain extent explain why coronary pain is generally absent in acute myocarditis or in chemically induced hypoxia *e g* by carbon monoxide (15 28)

Coronary and myocardial lesions in different types of anamnestic symptoms of infarction

As mentioned patients with a history of infarction were divided into two groups according to whether the course was typical or atypical and each group was subgrouped according to whether the infarction was previous or recent (Table XX c)

Among the cases where the symptoms satisfied Rose's criteria of previous infarction myocardial scars were found in 41 % (7 cases) Two subjects had a fresh infarction which had thus produced only insignificant symptoms Seven subjects (41 %) had no myocardial changes and 3 of these had no coronary stenosis either Thus this group included 35 % with a false positive diagnosis

The group with atypical previous infarction consisted of only 6 cases including 5 with myocardial scars after infarction but in 1 case there were neither myocardial nor coronary changes The group was too small to warrant any conclusions

The cases with recent infarction with a typical course were 3 times as common as those with an atypical course Of the 12 cases with an atypical course recent myocardial lesions were found in 5 scars after infarction in 3 and no myocardial changes in 4 None of these 4 subjects had severe coronary changes The diagnosis in these 4 cases was thus falsely positive Of the 35 cases in the group with typical anamnesis of recent infarction fresh myocardial changes were found in 20 Six cases had only myocardial scars or diffuse fibrosis Of those 6 without myocardial changes 2 had recent coronary arterial thrombosis 1 severe stenosis while 3 showed no coronary changes

The 2 cases with fresh coronary thrombosis had a clinical picture resembling that seen during an actual

attack of myocardial infarction but no infarct was demonstrable not even on microscopical examination This suggests that demonstrable myocardial changes had not had time to develop This is a source of error that should be borne in mind when considering the use of myocardial changes as criteria of IHD at autopsy After correction for this source of error and the exclusion of 3 cases with uncertain *post mortem* data it was found that 69 % of the cases with an anamnesis of recent typical infarction had expected myocardial changes, while 9 % (3 cases) had neither coronary nor myocardial changes In these 3 cases then the diagnosis had been false positive

Of those with combined previous and recent anamnestic infarcts good agreement was found with the post mortem findings

A question of interest in the evaluation of the method is whether these anamnestic criteria have the same validity for men as for women This was analyzed and the results are given in Tables XXII and XXIII The cases with angina pectoris and those with anamnestic symptoms of infarction were judged separately irrespective of any coexisting infarction anamnesis or angina pectoris In the various subgroups with anamnestic symptoms of IHD there was an unsignificant tendency for more false positive diagnoses among the women

Comments

Of interest in the elaboration of the epidemiological method and various problems related to IHD is that the cause of angina pectoris is believed by some investigators to be partly different from that of myocardial infarction Angina pectoris is believed to be mainly secondary to atherosclerosis while myocardial infarction is supposed to be a thrombotic complication and the two diseases would appear in different age groups and social classes (41) Moreover myocardial infarction has been described as responsible for the increase in the frequency of IHD in recent decades while the frequency of angina pectoris is believed not to have increased so substantially (41) This is an interesting hypothesis especially because if it is correct it would justify a differentiated elaboration of preventive measures But the results presented above showed that the two groups overlapped considerably in respect of vascular changes The above hypothesis will be tested in further detail in a future paper

The results set forth in this chapter also showed that the use of only myocardial changes as *post mortem* evidence of IHD introduces only a slight error in this series This applies to both angina pectoris and previous and recent infarctions In the following arterial changes will therefore be analysed only in special cases

Table XXII Myocardial changes demonstrated at autopsy in different types of angina pectoris in males and females

	No angina pectoris anamnesis	Atypical angina pectoris	Typical angina pectoris	Status anginosus
a *Males*	399	37	98	2
No myocardial changes	281 (70 %)	10 (27 %)	16 (16 %)	—
Recent infarction ± rupture	16 (4 %)	2 (5 %)	11 (11 %)	1
Myocard scar > 1 cm	55 (14 %)	16 (43 %)	31 (32 %)	—
Advanced diffuse fibrosis	14 (3 %)	1 —	3 (3 %)	—
Recent infarction + myocard scar	31 (5 %)	5 (13 %)	25 (25 %)	1
Recent infarction + diffuse fibrosis	3 (1 %)	—	3 (3 %)	—
Myocard scar + diffuse fibrosis	6 (1 %)	3 (8 %)	[illegible] (8 %)	—
Recent infarction + myocard scar + diffuse fibrosis	3 (1 %)	—	1 (1 %)	—
Uncertain postmortem data	(11)	(3)	(2)	(1)
b *Females*	430	29	69	4
No myocardial changes	355 (80 %)	12 (41 %)	16 (23 %)	—
Recent infarction ± rupture	10 (2 %)	2 (7 %)	2 (3 %)	—
Myocard scar > 1 cm	35 (8 %)	6 (21 %)	16 (23 %)	1
Advanced diffuse fibrosis	13 (3 %)	2 (7 %)	4 (6 %)	—
Recent infarction + myocard scar	9 (2 %)	3 (10 %)	21 (30 %)	3
Recent infarction + diffuse fibrosis	2 —	1	2 (3 %)	—
Myocard scar + diffuse fibrosis	6 (1 %)	2 (7 %)	4 (6 %)	—
Recent infarction + myocard scar + diffuse fibrosis	—	1 (3 %)	4 (6 %)	—
Uncertain postmortem data	(14)	(1)	(4)	

Table XXIII Myocardial changes seen postmortem in cases with different types of anamnestic symptoms of infarction

	No infarction in anamnesis	Atypical previous infarction	Typical previous infarction	Atypical recent infarction	Typical recent infarction	Combination of previous and recent infarction
a *Males*	401	6	26	12	52	34
No myocard changes	292 (72 %)	1	7 (27 %)	—	5 (10 %)	—
Recent infarction ± rupture	4 (1 %)	—	1	6 (50 %)	19 (36 %)	1
Myocard scar > 1 cm	67 (17 %)	4	10 (38 %)	3 (20 %)	5 (10 %)	12 (35 %)
Advanced diffuse fibrosis	16 (4 %)	—	—	—	1	—
Recent infarction + myocard scar	11 (3 %)	1	4 (15 %)	1	20 (38 %)	15 (44 %)
Recent infarction + diffuse fibrosis	2	—	—	1	—	3 (8 %)
Myocard scar + diffuse fibrosis	10 (2 %)	—	4 (15 %)	1	1	[illegible] (5 %)
Recent infarction + myocard scar + diffuse fibrosis	[illegible]	—	—	—	1	1
Uncertain postmortem data	(9)	—	(2)	—	(3)	(4)
b *Females*	460	3	16	8	31	20
No myocard changes	370 (80 %)	2	8 (47 %)	4	2 (6 %)	—
Recent infarction ± rupture	6 (1 %)	—	1	1	7 (23 %)	—

Myocard scar>1 cm	52 (11 %)	—	4 (23 %)	—	2 (6 %)	2 (10 %)
Advanced diffuse fibrosis	15 (3 %)	—	2 (12 %)	—	—	—
Recent infarction + myocard scar	10 (2 %)	1	—	—	14 (45 %)	13 (65 %)
Recent infarction + diffuse fibrosis	—	—	—	—	3 (9 %)	2 (10 %)
Myocard scar + diffuse fibrosis	7 (1 %)	-	1	—	2 (6 %)	1 (5 %)
Recent infarction + myocard scar + diffuse fibrosis	—	—	—	1	1	2 (10 %)
Uncertain postmortem data	(3)	—	(11)	—	(2)	(2)

Relation between electrocardiographic changes and post mortem evidence of ischaemic heart disease

As previously mentioned classification groups based on the criteria of the Minnesota code were used. These groups were selected *aprioristically* against the background of results obtained in other investigations and considerations reported largely in association with the presentation of the Minnesota code (12, 13, 14, 17, 18 and 33). It would of course be of interest to study each subcriterion separately and on the basis of the results find out which combination best satisfies the demands of specificity and sensitivity in this series. It is intended to carry out such an analysis after conclusion of the modification of the Minnesota code in progress (5).

Various investigations have been published of *post mortem* findings in cases with different electrocardiographic changes. Most of the series consisted of selected cases with clinical signs of myocardial infarction and the electrocardiographic criteria are often not defined, which makes it difficult to compare the results. Because of this and the fact that the purpose of the present investigation was to judge electrocardiography as one of several instruments used in the epidemiological method for recording IHD, comparisons with previously published results have only been made when they could give further information or elucidate the results obtained. The present series was also biased not only because of the selection criteria used (see chapter I) but also because those with suspected IHD were probably examined more often with electrocardiography than those without. As a rule, however, electrocardiography had been done routinely.

Myocardial lesions in association with different types of electrocardiographic changes

In this section the series are grouped according to previous and recent electrocardiographic changes and according to sex (Table XXIX and XXX). In the assessments the figures referring to cases with recent myocardial infarction in the table of *previous* electrocardiographic changes are less important and those with old lesions are of less interest in the table showing *recent* electrocardiographic abnormalities.

Of interest in this comparison is the number of cases with myocardial infarction in the group with normal cardiograms, i.e. the number with false negative diagnosis which reflects the lack of sensitivity of the method. Further the number of false positive diagnosis, i.e. cases with electrocardiographic changes but without myocardial scars is of interest because it reflects the lack of specificity of the criterion.

Previous electrocardiographic changes

Of the cases with a normal electrocardiogram 16 % had myocardial scars. A significant sex difference was found in this respect: 21 % of the men with normal electrocardiograms had myocardial scars compared with 11 % of the women ($\chi^2 = 12.0$, fg 1, $p < 0.01$). But such a comparison does not give a correct picture of the sensitivity of the criterion because *post mortem* evidence of IHD was more common in the men than in the women. This means that if the criterion is equally sensitive for both sexes the group with normal electrocardiograms will comprise more cases with post mortem evidence of IHD in the men. This number should instead be referred to the total number with myocardial scars in either sex. Even then, however, the difference will be probably significant (34 % compared with 24 %, $\chi^2 = 3.85$, fg 1, $p = 0.05$). The criteria in question are therefore probably more sensitive for women.

In the group with electrocardiographic changes in the form of conduction disorders and arrhythmias there were many individuals without myocardial scars (60 %). But this group included more cases with myocardial scars than did the group with a normal electrocardiogram, especially among the women. The criterion is thus fairly nonspecific. If this criterion is not accepted as a sign of IHD, more cases with real IHD will be referred to those who are judged as not having IHD according to electrocardiographic criteria and thereby further decrease the sensitiveness of the method. As previously mentioned the method of selection of the present series probably influenced the validity of this criterion because the number of cases treated with digitalis and the shift in electrolytes was greater than it would have been in a more representative sample of the population.

Of the subjects with previous electrocardiographic signs of infarction 37 % showed no myocardial scars and most of these had a normal myocardium (23 %). A significant difference was found between the sexes in this respect. In the males myocardial scars were more common ($\chi^2 = 10.3$, fg 1, $p < 0.01$). This criterion of old myocardial damage was thus less specific for women than for men in the present series.

The cases with left bundle branch block and low voltage were assigned to a special group. Only a few cases with low voltage were included and therefore the group may broadly speaking be regarded as consisting of subjects with leftsided bundle branch block. This group is important be

Table XXIV Myocardial changes seen postmortem in cases with different types of *previous* electrocardiographic changes

	Normal ECG	Not normal but no signs *of infarction* VI 1—4 VII 2—3 VIII 0—9 IX 2—9	Infarction pattern of ant and/or lat wall I 1—2 V 1—2 I 3/V [illegible]	*Infarction* pattern of post wall I 1—2 V 1—2 I 3/V [illegible]	Left bundle branch block or diffuse myocard changes VII 1 IX 1
a *Males*	293	123	46	33	46
No myocard changes	*197 (87 %)*	74 (60 %)	9 (20 %)	4 (12 %)	14 (30 %)
Recent infarction ± rupture	20 (7 %)	2 (2 %)	1	2 (6 %)	5 (11 %)
Myocard scar>1 cm	44 (15 %)	28 (23 %)	20 (43 %)	9 (27 %)	11 (24 %)
Advanced diffuse fibrosis	11 (4 %)	8 (6 %)	1	—	2 (4 %)
Recent infarction + myocard scar	14 (5 %)	5 (4 %)	10 (22 %)	12 (36 %)	9 (20 %)
Recent infarction + diffuse fibrosis	[illegible] (1 %)	—	1	2 (6 %)	1
Myocard scar + diffuse *fibrosis*	4 (1 %)	5 (4 %)	4 (8 %)	3 (9 %)	[illegible] (4 %)
Recent infarction + myocard scar + *diffuse* fibrosis	1	—	—	1	2 (4 %)
Uncertain postmortem data	(8)	(1)	(3)	(3)	(2)
b *Females*	293	134	28	23	44
No myocard changes	238 (81 %)	[illegible] (63 %)	*13 (46 %)*	8 (35 %)	14 (32 %)
Recent infarction ±					

Advanced diffuse fibrosis	14 (5 %)	5 (4 %)	2 (7 %)	2 (9 %)	2 (5 %)
Recent infarction + myocard scar	11 (4 %)	9 (7 %)	5 (17 %)	4 (17 %)	9 (21 %)
Recent infarction + diffuse fibrosis	1	—	1	1	2 (5 %)
Myocard scar + diffuse fibrosis	—	5 (4 %)	1	4 (17 %)	2 (5 %)
Recent infarction + myocard scar + diffuse fibrosis	—	—	2	1	—
Uncertain postmortem data	(8)	(5)	(9)	(—)	(—)

cause it is large 47 % had no myocardial scars and most of these had no myocardial changes (30 %) No difference with sex was found ($\chi^2 =$ 0 20 fg 1 p > 0 05) The criterion is less specific than the electrocardiographic signs called infarction pattern (page 30) ($\chi^2 = 4$ 93, fg 1, p < 0 05) but as expected much more specific than conduction disorders and arrhythmias ($\chi^2 = 57$ 8 fg 1, p < 0 01)

Recent electrocardiographic changes

The cardiographic criteria given appear to be more sensitive for recent than for old myocardial damage Thus of all the men with recent infarction only *10 % belonged to the* group with a normal electrocardiogram (Table XXV) The corresponding figure for women was 8 % The criteria comprising conduction disorders arrhythmias and S—T depression are as expected non specific signs of recent myocardial injury with 8 % having recent infarctions in both sexes which is slightly higher than in the group with normal electrocardiograms But because of the large total number in these groups the sensitivity is considerably reduced if these cases are assigned to the group not judged as having myocardial damage on the basis of the appearance of the electrocardiogram The number of undiagnosed recent infarctions then increases to 30 % in the men and to 35 % in the women Signs of local myocardial lesions infarction pattern appear to be rather specific with false positive diagnosis only in 17 % of the men and

Table III Myocardial changes seen postmortem in cases with different types of *recent* electrocardiographic changes

	Normal ECG	Not normal but no signs of infarction VI 1—4 VII 2—3 VIII 0—9 IX 2—9	S—T depression IX 1	Infarction pattern of ant and/or lat wall I 1—2 IV 4 V 1—2 I 3/V 3	Infarction pattern of post wall I 1—2 IV 4 V 1—2 I 3/V 3	Left bundle branch block or diffuse myocard changes VII 1 IX 1
a *Males*	180	97	150	42	35	24
No myocard changes	138 (74 %)	54 (56 %)	83 (55 %)	8 (19 %)	3 (9 %)	9 (37 %)
Recent infarction ± rupture	5 (3 %)	—	5 (3 %)	9 (21 %)	10 (29 %)	4 (16 %)
Myocard scar > 1 cm	29 (16 %)	31 (32 %)	37 (25 %)	6 (14 %)	4 (11 %)	4 (16 %)
Advanced diffuse fibrosis	8 (4 %)	3 (3 %)	8 (5 %)	—	1	—
Recent infarction + myocard scar	3 (2 %)	3 (3 %)	7 (5 %)	17 (40 %)	15 (43 %)	5 (21 %)
Recent infarction + diffuse fibrosis	—	2 (2 %)	1	1	1	1
Myocard scar + diffuse fibrosis	2 (1 %)	3 (3 %)	10 (7 %)	1	1	—
Recent infarction + myocard scar + diffuse fibrosis	1	1 (1 %)	—	—	—	1
Uncertain postmortem data	(6)	(5)	(2)	(2)	(3)	(—)
b *Females*	185	70	191	27	24	31
No myocard changes	159 (86 %)	39 (56 %)	137 (71 %)	7 (26 %)	2 (8 %)	12 (39 %)
[illegible]		2 (1 %)	6 (3 %)	7 (26 %)	3 (13 %)	2 (6 %)

Myocard scar>1 cm	11 (6 %)	12 (17 %)	33 (17 %)	2 (7 %)	1	7 (23 %)
Advanced diffuse fibrosis	9 (5 %)	8 (12 %)	6 (3 %)	—	1	—
Recent infarction+ myocard scar	3 (2 %)	5 (7 %)	6 (3 %)	9 (33 %)	10 (67 %)	6 (19 %)
Recent infarction+ diffuse fibrosis	—	1	—	—	1	2 (6 %)
Myocard scar+diffuse fibrosis	[illegible] (1 %)	[illegible] (3 %)	6 (3 %)	1	—	2 (6 %)
Recent infarction+ myocard scar+ diffuse fibrosis	—	—	—	1	—	—
Uncertain postmortem data	(—)	(—)	(10)	(—)	(—)	(3)

27 % of the women Left bundle branch block was as expected less specific it gave a false positive diagnosis in 55 % of the men and 68 % of the women In none of these groups was the numerical difference between men and women statistically significant

Anamnesis and electrocardiogram in epidemiological diagnosis of ischaemic heart disease

In epidemiological studies electrocardiographic signs have been recommended as more reliable and more objective than anamnestic criteria (35). It is of course important for the evaluation of the epidemiological method to know what significance should be attached to differences between the two methods since they are often applied simultaneously. In the present section the distribution of post mortem findings of IHD was studied in the following groups with different combinations of electrocardiographic changes and history, namely

1. without anamnestic symptoms of IHD and with a normal electrocardiogram
2. with anamnestic symptoms of IHD and corresponding electrocardiographic changes
3. with anamnestic symptoms of IHD but with a normal electrocardiogram
4. without anamnestic symptoms of IHD but with electrocardiographic changes of the type seen in myocardial infarction

The results are given in Tables XXVI a and b

Signs according to the Minnesota code I 1—2, IV 4 V 1—2, I 3/V 3 and VII 1 and IX 1 which mean Q and QS pattern, S—T increase, pathological R wave progression, T wave change left bundle branch block and low QRS amplitude, were accepted as criteria of myocardial damage. As previously shown S—T depression conduction disorders and arrhythmia are not very specific and cases with these changes were excluded from the present analysis. As this exclusion affects the sensitivity and the specificity evaluation, only the frequency of correct and false positive diagnosis was studied

The myocardium in subjects with agreement between anamnesis and electrocardiogram

Of the individuals with a normal electrocardiogram and without symptoms of IHD, 89 % showed no morphological signs of IHD. If diffuse fibrosis is not taken as a sign of IHD, the number without IHD in this group will be 93 %. In those cases where both the anamnesis and the electro

Table XXII Myocardial changes seen post mortem in cases with *different combinations* of electrocardiographic changes and *anamnestic symptoms of IHD*

a Agreement between anamnesis and ECG

	Without anamn sympt and normal ECG	Previous infarction in anamnesis + ECG changes of previous infarction	Recent infarction in anamnesis + ECG changes of recent infarction	Previous + recent infarction in anamnesis + ECG changes of previous and recent infarction	Angina pect without infarction in anamnesis + ECG signs of S—T depressions + previous infarction
Total	251	75	100	29	9
No myocard changes	224 (89 %)	8 (11 %)	6 (6 %)	—	1
Recent infarction	2 (1 %)	2 (3 %)	25 (23 %)	—	—
Myocard scar > 1 cm	13 (5 %)	22 (29 %)	9 (8 %)	2 (7 %)	4
Diffuse fibrosis	9 (4 %)	—	—	—	—
Recent infarction + myocard scar	1	27 (36 %)	55 (50 %)	21 (72 %)	1
Recent infarction + diffuse fibrosis	—	5 (7 %)	7 (6 %)	4 (14 %)	—
Scar + diffuse fibrosis	1	8 (11 %)	4 (4 %)	—	1
Recent infarction + scar + diffuse fibrosis	1	1 (1 %)	3 (3 %)	2 (7 %)	—
Uncertain postmortem data	(5)	(7)	(6)	(3)	—
Agreement between expected and demonstrated autopsy findings	89 %	80 %	92 %	79 %	75 %

Table XXVI b. Myocardial changes seen postmortem in cases with anamnestic symptoms of IHD but normal ECG and in cases with ECG changes of myocardial infarction but without anamnestic symptoms of IHD

	Recent infarction in anamnesis + normal ECG	Previous infarction in anamnesis + normal ECG	Previous + recent infarction in anamnesis + normal ECG	Angina pectoris + normal ECG	Recent infarction in ECG but without anamnestic symptoms	Previous infarction in ECG but without anamnestic symptoms	Previous + recent infarction in ECG without anamnestic symptoms
Total	22	29	4	16	29	47	16
No myocard. changes	2 (9 %)	10 (34 %)	—	7 (44 %)	21 (72 %)	30 (64 %)	12 (75 %)
Recent infarction	4 (18 %)	1 (3 %)	—	—	—	1 (2 %)	—
Myocard. scars > 1 cm	8 (36 %)	9 (31 %)	2	8 (50 %)	4 (14 %)	5 (11 %)	2 (12 %)
Diffuse fibrosis	1 (5 %)	2 (7 %)	—	1 (6 %)	—	3 (6 %)	—
Recent infarction + myocard. scar	3 (14 %)	6 (21 %)	1	—	3 (11 %)	4 (9 %)	2 (12 %)
Recent infarction + diffuse fibrosis	2 (9 %)	—	—	—	—	1 (2 %)	—
Scar + diffuse fibrosis	—	1 (3 %)	1	—	—	2 (4 %)	—
Recent infarction + scar + diffuse fibrosis	2 (9 %)	—	—	—	—	1 (2 %)	—
Uncertain postmortem data	(1)	(3)	—	(5)	—	(2)	—
Agreement between expected and demonstrated autopsy findings	50 %	55 %		50 %	11 %	26 %	12 %

cardiogram argued for infarction expected changes were found in 76 and 80 %. Thus as supposed the frequency of false positive and false negative diagnoses in these groups was low.

The myocardium in subjects with poor agreement between anamnesis and electrocardiogram

Among individuals with anamnestic symptoms of IHD but with a normal electrocardiogram post mortem evidence of IHD was found in 50 and 55 % in case of recent respectively previous history of infarction (Table XXXI b). When only electrocardiographic signs of myocardial damage had been noted and the patient had denied symptoms of IHD expected myocardial changes were found in only 11—26 % at post mortem. This difference was statistically significant ($\chi^2 = 8.7$ fg 1, $p < 0.01$, $\chi^2 = 6.8$ fg 1, $p < 0.01$).

The above mentioned differences in diagnostic efficacity between anamnesis and electrocardiogram are artificially low because only the expected myocardial changes were estimated. Of those with anamnestic symptoms of only recent infarction many showed myocardial scars as the only finding or in combination with fresh infarction (59 %) and of those with a history of only previous infarction recent infarction was found post mortem in 24 %. This reflects the well known clinical experience that infarction has a strong tendency to recur. In addition further support was obtained for the previously demonstrated phenomenon that the clinical symptoms are liable to vary in type from time to time in one and the same patient. The course may sometimes be so insidious that the symptoms are not regarded as referable to the heart or are simply forgotten, while a later infarction in the same individual may run a dramatic course, an observation reported also by Snow (17).

Comments

The results provide a basis for certain considerations concerning the sensitivity and specificity of the method. If anamnestic signs of IHD combined with a normal cardiogram are accepted a further 62 cases with post mortem evidence of IHD would be diagnosed while 19 without IHD would be accepted as IHD. This means an increase of the sensitivity without any essential reduction of the specificity. Further if electrocardiographic signs of infarction (as defined above on page 62) in subjects who had denied symptoms of IHD were accepted it would mean the diagnosis of a further 28 cases with IHD demonstrated post mortem and 63 falsely accepted as IHD. A moderate increase in sensitivity would thus imply a relatively large loss of specificity.

The results show further that it is possible not only to obtain a group with a true high frequency of the disease but also, which is probably of interest from an epidemiological point of view, to distinguish a group which

is fairly free from the disease Even in this material it was possible to distinguish these extreme groups so that they comprised a relative large proportion of the series In addition a number of intermediate groups were obtained with IHD with varying degree of probability These intermediate groups consisted above all of individuals who had denied anamnestic symptoms of IHD but who were separated from this group because of electrocardiographic changes judged as fairly nonspecific

The results suggest that the anamnesis is the most important method in the diagnosis of IHD while electrocardiography as an independent method does not appear to contribute essentially to the firmness of the diagnosis It has however, great value as a supplementary method

Factors impairing sensitivity and specificity of the method

The factors reducing sensitivity and specificity of the method are referable to two groups of individuals those with a false negative diagnosis of IHD *i e* those in whom IHD was not diagnosed with anamnesis or electrocardiogram but in which autopsy showed myocardial infarction and those with a false positive diagnosis, *i e* those judged as having IHD from anamnesis and electrocardiogram but in whom autopsy showed no morphological signs of IHD Attempts were therefore made to find a method for correcting for these sources of error This was thought possible if certain characteristics might be demonstrable in individuals in these two groups—characteristics distinguishing them from the other cases in the group It appears reasonable *a priori* to assume that these two groups are not homogenous but are due to several aetiological factors which might produce manifestations separately or possibly in combination with one another

Cases without symptoms of IHD but with post mortem evidence of infarction (*silent infarction*)

The definition of silent infarction varies from author to author In the Framingham study (2) supervened electrocardiographic signs of myocardial lesions without anamnestic symptoms referable to the heart were taken as a sign of silent infarction In some post mortem investigations all cases in which the physician when requesting post mortem had not noted myocardial infarction but in which myocardial lesion was found were classified as silent (23 24)

In the present investigation myocardial infarction was said to be silent if at the time of the last admission to hospital the patients had denied having had symptoms referable to the heart and if he had been cared for previously at Malmö General Hospital no signs of IHD had been noted in his records This means either that the patients have had no symptoms interpreted as cardiac symptoms or that any symptoms they might have had were such that the patients did

not recall them. Neither should they have had any electrocardiographic abnormalities suggesting myocardial infarction according to the definition given in page 62. Recent myocardial infarction or myocardial scars more than 1 cm in diameter should further have been demonstrated post mortem. Cases with only diffuse myocardial fibrosis were not accepted.

In an epidemiological investigation these cases introduce a systematic source of error because they are assigned to the group judged as not having IHD. This means that when the group judged as having IHD is compared with that judged as not having IHD these cases tend to mask differences which might be related to IHD factors.

The reason why myocardial infarction does not always produce symptoms has been the subject of various investigations. The theoretical basis of the suggestions offered are however often less convincing owing to the lack of control series. Subjects with anamnestic symptoms of myocardial infarction verified post mortem would be suitable as controls. If another type of controls is used it will probably be difficult to decide whether differences, if any, were due to factors related to IHD or whether they were related to the fact that IHD in these cases was asymptomatic. In the present series the control cases used consisted of those which had had typical anamnestic symptoms of infarction (for definition see page 2a) and in which autopsy had revealed myocardial infarcts. The comparison was also limited to previous myocardial infarctions.

In previously published series two fundamentally different approaches were used in the search for the causes. Firstly, attempts were made to explain why the myocardial infarction ran silent, secondly it has been assumed that the symptoms were equally severe as in the overt cases but for some reason or other had not been noted or interpreted by the subject in the same way.

It has been tentatively suggested that these silent infarcts occur in the *silent zones* of the myocardium. But the fact that no relation has been found between the course and the site of the damage weighs heavily against this hypothesis (28). It has also been assumed that certain subjects have a *high pain threshold* (1, 2, 28) and thereby experience the pain in a different way. But this is difficult to bring in line with the fact that, as mentioned, one infarction in a person may be asymptomatic while a later one in the same person run a very dramatic course. Further it has been postulated that in *elderly individuals* the course of myocardial infarction is less dramatic than in young ones and that the former are more liable to forget a previous attack of pain (24, 36). But no difference in age distribution could be demonstrated (Fig. 3) between cases with silent infarction and those with infarction which has given clinical symptoms (here called symptomatic infarction) in the present series ($t = 0.42$, $p < 0.05$). Nor was any significant difference found when the

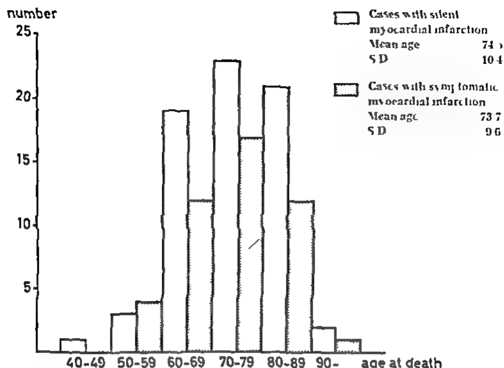


Fig 3 Age distribution in cases with myocardial infarction post mortem divided in anamnestic silent and symptomatic cases No significant difference in age or age distribution could be demonstrated

data were classed according to sex and compared in a corresponding way ($t = 0\,20$ $p > 0\,05$ $t = 0\,75$ $p > 0\,05$) The fact that no age difference could be found and that the numerical difference was so small facilitates further comparisons

It has also been assumed that silent infarctions occur mainly in patients with *arterial hypertension* This hypothesis is based on the fact that the individuals in series with silent infarction , usually have large hearts (21 24) In the present investigation however no difference in the frequency of hypertension was demonstrated between the two series (Table XXVII a) But a significant difference was found in the heart weight in both sexes (Table XXVII b) with a lower weight in cases with silent infarction than in the controls with symptoms on their infarction This seems to argue against the cited results (21 24) But it need not The difference may be due to differences in the control series When cases of silent infarction were compared with those without IHD it was found that also in the present series the heart weight was significantly increased in the men but not in the women If Evans et al (21) in

Table XXVII Comparison between hypertension variables in symptomatic infarction and in silent infarctions

	Males		Females	
	Symptomatic infarction	Silent infarction	Symptomatic infarction	Silent infarction
a *Diagnosis of hypertension*				
Normal blood pressure	21	22	1	16
High blood pressure complications not studied	5	8	4	5
High blood pressure without complications	2	—	1	—
High blood pressure only with enlarged heart	—	—	—	1
High blood pressure with enlarged heart and renal changes or changes in ocular fundi	4	6	[illegible]	8
Malignant hypertension	—	—	—	1
Incomplete data	(1)	(2)	—	(1)
Total	32 (33)	36 (38)	14	31 (32)
	$\chi^2=0.25$ f_n 2 $p>0.05$		$\chi^2=2.7$ fg 3 $p>0.05$	
b *Heart weight*	456.3 ± 127	365.8 ± 110	414.3 ± 112	354.5 ± 109
	t = 3.19**		t = 1.73*	
c *Thickness of left ventricular wall*	4.58 ± 1.26	4.58 ± 1.05	4.67 ± 0.93	4.40 ± 0.92
	t = 0.00		t = 0.54	
d *Thickness of right ventricular wall*[1]	3.77 ± 1.33	3.61 ± 1.61	3.67 ± 1.00	3.30 ± 1.17
	t = 0.44		t = 0.98	

[1] Not absolute values provisional values used

their evaluation of high heart weight compared it with normal heart weight in a series of mixed sex there results could be explained and would agree with those in the present investigation But they do not state how they calculated their reference heart weight or how large it was As mentioned above however differences between cases with and without IHD should reflect factors associated with IHD and not the type of course of IHD In the present series the heart weight in subjects with silent infarction was smaller than in those with symptomatic infarction This however does not necessarily mean that hypertension was more common in the latter Other important factors capable of influencing the weight of the heart are organic valvular disease bodybuild and physical training Only in one case was there a clinical history of valvular disease (aortic stenosis) among the cases with symptomatic infarction Among the cases with silent infarction were 2 with a clinical history of valvular heart disease

which had been interpreted as a combined aortic vitium and a congenital vitium respectively. Post mortem examination showed a further case of aortic stenosis among those with symptomatic infarction and the clinical diagnosis in the two above mentioned cases with silent infarction were verified. Even after these cases had been excluded there was a statistically significant difference in heart weight between the two groups. Thus it seems as if *organic valvular heart* disease did not cause the difference in heart weight.

It appears *a priori* to be reasonable to assume that the differences in blood pressure in the general circulation *may have been of significance in the* causation of the differences in heart weight. As mentioned however no statistically significant difference could be found in the frequency of arterial hypertension even if there was a weak tendency *for hypertension to* be more common among the women with symptomatic infarction. If those cases with a high blood pressure were excluded the difference in heart weight in the males would persist. In the *females a numerical difference* would also persist but it would not be statistically significant. If the difference *in heart weight is due to a high* blood pressure it should be reflected in differences in the thickness of the wall *of the left ventricle*. But no such difference was demonstrable (Table XXVII c). Nor could any difference be demonstrated in the thickness of the wall of the right ventricle (Table XXVII d). This reduces the validity of the significant difference in heart weight between the two groups. A third possible explanation of the difference in heart weight might be differences in body build and physical fitness which however were not studied.

Summing up no evidence was found in support of the assumption that hypertension should be a causal factor of silent infarction more than in overt infarction. Nor could any evi*dence be demonstrated for the* assumption that a difference in blood pressure is the cause of the increased heart weight in subjects with symptomatic infarctions compared with the subjects with silent infarction.

It has been claimed by some authors that there is no true difference in the clinical picture of patients with silent infarctions and of those with symptomatic infarctions and various reasons had been given why these cases *do not behave in the same way*. *One* factor might be that some individuals will not accept a symptom as a symptom of heart disease because it would mean the acceptance of a serious diagnosis (47). Such a hypothesis is how*ever impossible to test in the present* series. Further it has been claimed that heart pain can be concealed by impairment of the sensorium often because of a *cerebrovascular insult*. No difference could however be demonstrated in respect of *intra vitam* or *post mortem* evidence of cerebrovascular insult (Table XXVIII a, c and d). It has also been postulated that pain can be misinterpreted by the subjects who have some other chronic painful

Table XXVIII Cerebral vascular lesion and clinical and postmortem signs of athero sclerosis in cases with symptomatic and silent myocardial infarction

	Males		Females	
	Symptomatic infarction	Silent infarction	Symptomatic infarction	Silent infarction
Clinical data				
a Cerebral vascular lesion				
Without signs of vasc lesion	22	27	4	15
With signs of vascular lesion	10	11	10	16
Uncertain data	(1)	—	—	—
Total	32 (33)	38	14	31
			$\chi^2=1.54$ fg 1 $p>0.05$	
b Intermittent claudication				
Without signs of claudicatio	30	35	13	31
With signs of claudicatio or amputation	2	2	1	—
Uncertain data	(1)	(1)	—	—
Total	32 (33)	37 (38)	14	31
Postmortem data				
c Cerebral vessels				
Without signs of changes in cerebral arteries	27	31	8	20
Fresh or old thrombosis	4	—	4	11
Severe stenosis	1	2	2	—
Aneurysm or rupture	—	4	—	—
Uncertain data	(1)	(1)	—	—
Total	32 (33)	37 (38)	14	31
d Cerebral infarction				
Without cerebral infarction	25	28	5	16
Recent infarction	3	1	4	5
Old infarction	1	3	1	5
Status lacunaris	-	4	2	3
Combination	2	2	2	2
Uncertain data	(2)	—	—	—
Total	31 (33)	38	14	31
e Atherosclerosis	t p		t p'	
Aortic arch	3.03 ± 0.81	3.08 ± 0.79	3.39 ± 0.85	2.71 ± 0.67
	0.20 >0.05		2.22 <0.01	
Mid aorta	3.[illegible]6 ± 0.91	3.62 ± 0.86	4.31 ± 0.80	3.65 ± 0.92
	0.21 >0.05		2.21 <0.05	
Left desc cor artery	3.44 ± 0.82	3.21 ± 0.73	3.07 ± 1.49	2.41 ± 1.08
	0.90 >0.05		1.61 >0.05	
Left circ cor art	2.75 ± 1.09	2.51 ± 1.01	2.43 ± 1.34	1.90 ± 1.10
	0.80 >0.05		1.29 >0.05	
Right cor artery	2.91 ± 0.71	2.86 ± 1.06	2.50 ± 1.12	2.00 ± 1.21
	0.70 >0.05		1.20 >0.05	

t denotes Student's t test and p probability of deviation of t from nil is due to chance

Table XXIX Comparison regarding frequency of gallstone in cases with symptomatic and silent infarction

	Males			Females		
	Symptomatic infarction	χ^2	Silent infarction	Symptomatic infarction	χ^2	Silent infarction
Without anamnestic symptoms suggesting gallstone	26		36	7		26
With anamnestic symptoms suggesting gallstone or operated upon for gall stone	6		2	7		5
		0.20 fg 1 $p>0.05$			6.45 fg 1 $p<0.01$	
No gallstone at autopsy	14		24	3		11
Gallstone or status after operation for gallstone	18		13	11		20
		3.10 fg 1 $p<0.01$			0.88 fg 1 $p>0.05$	

disease the coronary pain then being referred to the usual sort of pain *e g gallstone or peptic ulcer* (15 28) But no difference was found regarding the frequency of anamnestic symptoms of ulcer and of evidence of ulcer post mortem (these data are not included in the tables) On the other hand differences were found in the frequency of gallstone (Table XXIX) The difference however weighed against the above hypothesis In subjects with silent infarction both in the men and the women anamnestic symptoms and post mortem findings of gallstone were less common than in the subjects with symptomatic cardiac infarction This numerical difference was statistically significant only regarding the frequency of gallstone found in the men post mortem and symptomatic gallstone in the women (Table XXIX) This difference can hardly be a manifestation of competing selective factors which go under the common name of Berkson's fallacy (6 7) but presumably represent a biologically meaningful relation

Other conditions of the heart such as *cardiac incompensation and auricular fibrillation* have been thought to be able to mask myocardial infarction (21 23 24) But no difference was found in the males or in the females regarding the number with cardiac incompensation ($\chi^2 = 0.40$ fg 1 $p > 0.05$, $\chi^2 = 2.0$ fg 1 $p > 0.05$) The problem of auricular fibrillation was not studied because the control cases were not suitable in this connection

In the present analysis it was shown that the heart weight is higher in

subjects with symptomatic cardiac infarction. This could not be explained by any difference in the frequency of valvular heart disease. Neither was any evidence produced for a high blood pressure being the cause of the differences. It is therefore possible that differences in body build may explain the difference in heart weight between the subjects with silent and with symptomatic infarctions. These bodybuild factors which can influence the heart weight have previously been shown to be related to the severity of atherosclerosis in the coronary vessels (22). Now if bodybuild factors are responsible for the difference in heart weight, one might expect a difference in coronary atherosclerosis in such a way that atherosclerosis was not so severe among cases with silent infarction. Such differences were also found in certain vascular areas. In women there was significantly more advanced atherosclerosis of the aortic arch and of the abdominal aorta and a systematic tendency not statistically significant for atherosclerosis of the coronary vessels to be more advanced in subjects with symptomatic infarction. No differences in these respects were found among the men (Table XXVIII c). It was of interest to test whether this difference could be assigned to the morphologically relevant changes, i.e. cases with severe stenosis and occlusions. It is clear from Table XXX and Fig. 4 that individuals with myocardial scars but without any IHD anamnesis less often had severe coronary lesions. This result can, as mentioned above, be due partly to the assumption that an earlier thrombus in the coronary vessels had retracted or become recanalised and lined with intima (38, 39, 40). Also the results in other published investigations argue for atherosclerosis and thrombotic complications being less common in cases with silent infarctions. On careful post mortem examination at which special attention was given to the size of the infarctions it was shown that, unlike large scars, scars less than 2 cm appeared not to be so intimately related to the atherosclerosis of the coronary vessels as the large scars (20, 48). In another post mortem examination of an infarction series in which cases with and without occlusion of the coronary vessels were compared, the myocardial lesions in the cases without occlusions tended to be small, isolated and not associated with such striking clinical symptoms as the cases with thrombosis (12).

These results support the hypothesis that in patients with silent infarction the lesion to some extent might less often be caused by atherosclerosis and thrombotic complications as in cases with symptomatic infarction. Also the relation between gallstone and the symptomatic infarction suggests, irrespective whether this relation is a manifestation of two symptoms of a disorder of the cholesterol metabolism or some other condition, that the silent infarctions to a certain extent have different aetiology from that of the clinically symtomatic cases.

The pathogenesis of these myocardial scars without clinical symptoms is of course of interest. They may be

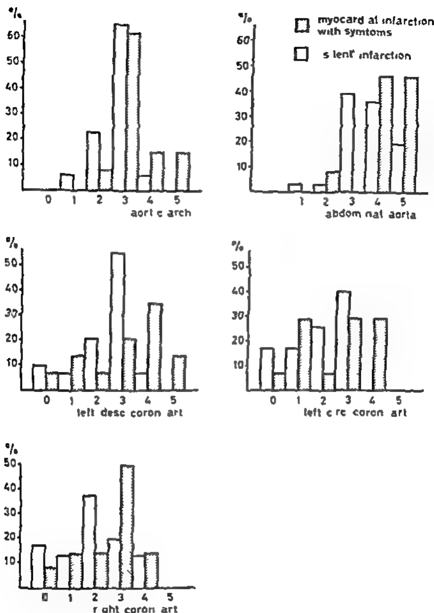


Fig 4 Grade of atherosclerosis in cases with symptomatic and silent infarction in females

sequelae after acute intimal swelling or bleedings which have regressed (38) They might also be scars after myocarditis But such scars are rarely of this localized type (53) No evidence were obtained that these changes were of rheumatic type or conditions after septic emboli The possibility of metabolically induced necrosis (30 53) remains

7 le XXX (ro ars l o s s w l lff r t pt s T I II l f rn l j l morle el lel

	W l t IIID l	Atyp l n a		T p n		At p al nfar t o s p le	Typ cal infarct o ej le
		W l l f t	W l t l f r t	W l l r t	W t o t n n t r t a		
[illegible]	( )	1	14(0	1 (1)	1 ll o)	4(33)	0(0 %)
[illegible]	11 1	4	1 14	(	15(39 o)	o(41	0(0 %)
Ste s s+o cl	8(8	(1 o	3 11	3(	7 (9)	1(10 %)	0(0 %)
Only c l	1 (11 %)	8 4)	7(	1)	3 (9 o)	2(18 o)	18(40 o)
T tal	100	16	74		9	1	4
1 certa cases	(14)	(	( )	(1	(1)	(1)	

1 χ^2 l str l t of c ro ars le o s s cases th s le t nfarct n compare l w th cases th sym plom at c farct on a l ang n pector s of l ff rent types In tl e χ^2 a alys s Yates correct on as use l when tl e n l er n any of tl e cells as less tl an

	I farct + ang a pector s	Atyp cal ang na pectoris	Typ cal ang na pector s	Infarct on w tl o t ang na pectoris
S lent nfarct o n	63 6**	4 7	14 9**	32 3**

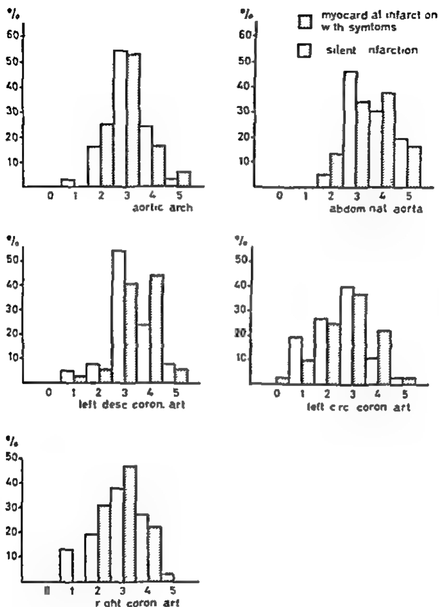


Fig 5 Grade of atherosclerosis in cases with symptomatic and silent infarction in males

These results may imply a limitation of the possibility of using myocardial scars as a definite sign of IHD if the term IHD is to mean sequelae after atherosclerotic and thrombotic conditions of the heart. Myocardial scars alone as a criterion would thus probably result in too many false negative diagnoses. The frequency of false positive diagnoses would probably be affected less. Certain observations suggest that these errors of the method can be corrected to a certain extent if only scars larger than 3 cm in diameter be accepted as a criterion of IHD of this type (20, 48), but assessment of the error of such a procedure requires further investigation.

Pathogenic factors discussed in previous publications on silent infarctions were not found to be of any significance in the present series, which may to a certain extent be due to the fact that the term is defined in a different way with the result that the series differ in composition. No characteristics over and above those possibly related to a lower degree of coronary atherosclerosis could be demonstrated in cases of silent infarction.

Cases with symptoms of ischaemic heart disease but without post mortem evidence of the condition

In these cases there were anamnestic symptoms simulating those of IHD. In an epidemiological investigation these cases introduce a systematic source of error with reduction of the specificity of the method. This implies a tendency to mask differences related to real IHD.

A systematic analysis of these cases in all symptom groups separately would be of interest, but there were not enough cases of this type in the groups with anamnestic symptoms of infarction to allow conclusive results (Table XX). Moreover, it was not considered advisable to take together cases from different symptom groups because of the possibility of different causal factors simulating different symptoms of IHD. The group with false positive diagnosis in the individuals with angina pectoris was judged as large enough for analysis. Therefore, this analysis was confined to cases with clinical symptoms judged as angina pectoris but without coronary or myocardial changes at autopsy.

Suitable as controls are age and sex matched subjects without post mortem signs of IHD. The controls were selected in the following way. For each subject with angina pectoris like symptoms we accepted next autopsied individual of the same sex and at most 3 years younger or older at the time of death and who had no clinical signs of IHD or no myocardial or coronary changes compatible with IHD.

One might a priori expect cases of atypical angina to be overrepresented among those cases with symptoms falsely interpreted as IHD but no significant difference was found between cases with typical and atypical angina pectoris regarding the frequency of

false positive diagnosis ($\chi^2 = 1.41$, f.g. 1, $p > 0.05$).

Mainly two mechanisms might be imagined in the causation of angina pectoris-like symptoms, namely extracardiac changes producing recurrent pain resembling that of angina pectoris and pathological conditions involving the heart and thereby precipitating symptoms conceived as angina pectoris. Among the extracardiac causes diseases of the gallbladder and the stomach may be considered, the heart by its gastrocoronary and cholangio-coronary reflexes (15) then acting as a "resonator" of the pain in these organs (28). Further, it has been shown that pulmonary emboli are often conceived as IHD (23). Also pneumonia, emphysema and cor pulmonale might produce IHD-like symptoms and a segment syndrome due to compression of the root of a spinal nerve can produce pain simulating that of IHD. There are other less common diseases from which IHD pain must be differentiated.

Hypertension and valvular heart disease by their effect on the heart may produce pain difficult to distinguish from true angina pectoris (46). By a partly different mechanism anaemia is also believed to cause angina pectoris (54) but probably only in the presence of a coronary vascular stenosis (15).

Conditions capable of simulating angina pectoris

Tables XXXI gives a comparison between the patients with IHD-like symptoms and controls of some of the conditions believed to be capable of simulating angina pectoris. The frequency of clinical signs and post-mortem evidence of peptic ulcer and of gallstone and autopsy findings of pulmonary embolism and pneumonia was studied. Neither among the men nor among the women was any difference found. It was astonishing that the number of cases with pulmonary embolism was so low among the subjects with symptoms simulating IHD. This might, however, have been due to the fact that a comparison was made of angina pectoris-like symptoms. It is probable that pulmonary emboli more often simulated myocardial infarction.

Thus no evidence was produced for the possibility that peptic ulcer and gallstone, pulmonary embolism and pneumonia had produced symptoms diagnosed as angina pectoris in this series.

Hypertension and valvular heart disease as cause of angina pectoris-like symptoms. It is quite likely that other diseases than atherosclerosis involving the heart or the circulation can produce symptoms resembling angina pectoris. Cases of hypertension and valvular heart disease producing such symptoms have often been described (46, 33). These observations agree with clinical experience. Also in the present series cases with hypertension and enlarged hearts were overrepresented among patients with symptoms simulating angina pectoris. In the males there were 30 % with hypertension compared with 16 % in the

Table XXXI Frequency of gallstone and gastric or duodenal ulcer in patients with angina pectoris like symptoms and in controls

	Males		Females	
	Angina like symptoms	Controls	Angina like symptoms	Controls
a *Gastric or duodenal ulcer*				
Clinical data				
No anamnestic symptoms	20	17	19	19
Symptoms of gastritis	1	1	—	—
Clin. diagn. gastric ulcer	—	2	1	1
Duodenal ulcer	—	2	1	1
Melena UNS	2	1	1	1
Uncertain data	—	—	—	—
	$\chi^2 = 1.21$	$p > 0.05$		
Postmortem data				
No signs of ulcer	20	13	16	16
Recent ulcer	2	4	1	1
Scar after ulcer	1	4	2	2
Other gastro intestinal diseases	—	2	3	3
	$\chi^2 = 2.08$	$p > 0.05$		
b *Gallstone*				
Clinical data				
No anamnestic symptoms	22	21	13	17
Clinical signs of gallstone	1	2	[illegible]	5
Postmortem data				
No gallstones	14	15	8	[illegible]
Gallstones	9	9	14	16
c *Pulm. embolism and pneumonia*				
Without signs of pulm. emb.	[illegible]	20	18	19
With signs of pulm. emb.	4	3	4	3
Without signs of pneumonia or severe bronchitis	14	17	15	11
With signs of pneumonia or severe bronchitis	9	6	7	11
			$\chi^2 = 1.50$	$p > 0.05$
d *Valvular heart defect clinically diagnosed*				
No	18	23	20	22
Yes	4	—	2	—
Demonstrated post mortem				
No	[illegible]	23	19	22
Yes	4	—	4	—

Table XXXII Various hypertension variables in cases with angina pectoris like symptoms and in controls

	Males		Females	
	Angina like symptoms	Controls	Angina like symptoms	Controls
a. *Diagnosis of hypertension*				
Normal blood pressure	15	17	7	10
High blood pressure complications not studied	1	2	7	8
High blood pressure without complications	—	—	[illegible]	—
Only with enlarged heart	1	—	1	1
With enlarged heart and renal changes or changes in ocular fundi	[illegible]	3	4	[illegible]
Malignant hypertension	—	—	—	—
Incomplete data	—	1	1	1
Total	23	22 (23)	22	22
b *Heart weight*	439 ± 135 t = 1.84	361 ± 152 $p < 0.05$	300 ± 85 t = 0.52	286 ± 93 $p > 0.05$
c *Thickness of left ventricular wall*[1]	4.73 ± 1.13 t = 2.97	3.73 ± 1.15 $p < 0.01$	4.48 ± 0.60 t = 1.37	4.14 ± 1.00 $p > 0.05$
d *Thickness of right ventricular wall*[1]	3.77 ± 1.54 t = 2.02	2.95 ± 1.12 $p < 0.05$	3.29 ± 0.75 t = 2.50	2.71 ± 0.72 $p < 0.01$

[1] *Not absolute values provisional values used*

controls, the corresponding figures for the females being 23 % and 16 % These differences were however, not statistically significant Further the cases with angina pectoris like symptoms included [illegible] with valvular heart disease which was not seen in any of the controls If, in these cases the hypertension and valvular heart disease had contributed to the symptoms referable to the heart they should have influenced the weight of the heart Most of the males with symptoms resembling angina pectoris really had heavier hearts (Table XXXII) This difference can be referred to both the thickness of the wall of the left ventricle *and* that of the right ventricle (Tables XXXII a b and c) In the females the difference was found only in the thickness of the right ventricular wall (Table XXXII c) To check whether cases with valvular heart disease and hypertension were responsible for the higher heart weight and the difference in the thickness of the ventricular wall these cases were excluded After the

4 cases with organic heart disease had been excluded from the male group, the difference in heart weight was reduced and no longer statistically significant ($t=1.27$, $p>0.05$), but still probably significant regarding the thickness of the left wall ($t=2.32$, $p<0.05$) and the right wall ($t=1.87$, $p<0.05$). When also male cases that had a high blood pressure were excluded from this group and from the controls, the numerical difference was only small regarding the heart weight ($t=0.20$, $p>0.05$) and the thickness of the right wall was numerically, on the average, somewhat larger among the controls. Yet the difference regarding the left wall ($t=1.72$, $p<0.05$) was still probably significant. After the 4 cases with valvular heart disease in the female group had been excluded the difference in the thickness of the right wall persisted ($t=2.40$, $p<0.05$). When also the cases with hypertension were excluded from both the cases with angina pectoris like symptoms and from the controls in females, no difference in the thickness of the right ventricular wall persisted ($t=0.0$, $p>0.05$).

The larger number of cases with valvular heart disease and hypertension in the group with symptoms interpreted as signs of angina pectoris thus seems to be able largely to explain the difference in heart weight and in the thickness of the ventricular walls shown on comparison with the controls. Since the cases with valvular heart disease and hypertension had such a strong effect on the mass of the heart muscle it was tempting to assume that this could explain the angina pectoris like symptoms in these cases. This pain was considered to be due to the fact that the increased demand for blood by the enlarged heart could not be satisfied in cases of valvular heart disease and hypertension with the generally increased demands on the oxygen transport that is present in these cases (15).

As is well known, aortic stenosis causes angina pectoris like symptoms. This conception is supported by the types of clinically diagnosed valvular heart disease among subjects with angina pectoris like symptoms. Of the 6 cases, 4 had aortic valvular disease including stenosis in 2, insufficiency in 1 and combined disorder in 1, 1 had mitral stenosis and 1 an unclassifiable type. Aortic valvular disease appears to be overrepresented on comparison of the distribution between clinically diagnosed aortic and mitral valvular disease in the entire series. The difference was, however, not statistically significant ($\chi^2=0.8$, f_h 1, $p>0.05$). At autopsy the 4 cases of aortic disease and mitral disease were verified but 3 cases of mitral disease were also discovered, which makes the distribution between different types of valvular diseases equal. It agrees with the distribution of the disease of the aortic and mitral valves in the entire series found *post mortem*.

Among the men with angina pectoris like symptoms there was also a systematic but not significant tendency for the coronary atherosclerosis to be more advanced than in the controls (Table XXXIII). The difference was reduced considerably when cases with hypertension were excluded, which suggests that a high blood pressure can have produced an effect in this respect too. As mentioned above

Table XXVIII Clinical signs of cerebral vascular lesion and postmortem evidence of athero sclerosis in cases with angina pectoris like symptoms and in controls

	Males		Females	
	Angina pectoris like symptoms	Controls	Angina pectoris like symptoms	Controls
a *Clinical signs of cerebral vascular lesions*				
Without signs of cerebral vascular lesion	17	15	13	15
With signs of cerebral vascular lesion	6	7	8	7
Uncertain data	—	1	1	—
Total	23	23 (23)	21 (22)	22
b *Cerebral infarction seen post mortem*				
No cerebral infarction	19	21	14	18
Recent infarction	3	2	5	—
Old infarction	1	—	1	—
Status lacunaris	—	—	—	3
Combinations	—	—	2	1
Uncertain data	—	—	—	—
Total	23	23	22	22
c *Atherosclerosis*				
Aortic arch	2.30±1.00	2.56±0.79	3.18±0.50	2.95±0.85
	t=0.34 p>0.05		t 0.76 p>0.05	
Abd aorta	3.13±1.20	2.96±0.79	3.82±0.77	3.81±0.85
	t=0.34 p>0.05		t=0.03 p>0.05	
Left desc cor art	2.65±1.28	2.00+1.12	2.82+0.64	2.67±0.93
	t=1.12 p>0.05		t 0.45 p>0.05	
Left circ cor art	2.00±1.41	1.50±1.13	2.09±1.17	1.95+0.89
	t=1.21 p>0.05		t=0.30 p>0.05	
Right cor art	2.26+1.26	1.43+1.25	2.50±1.00	2.09±1.18
	t=1.44 p>0.05		t 0.80 p>0.05	

coronary atherosclerosis was never so advanced as to cause substantial stenosis of the coronary arteries

Comments

The results show that certain common painful conditions in the chest and in the upper part of the abdomen are not especially common in patients with symptoms falsely interpreted as angina pectoris On the other hand there was reason to assume that sub jects with valvular heart disease and hypertension with cardiac enlargement contributed to the symptoms simulating angina pectoris in the absence of severe coronary stenosis

or myocardial scars after infarction In this series, then this might have been so in 11 of the 23 cases in the males and in 9 of 22 cases in the females As to the remaining cases nothing was found to explain the angina pectoris like symptoms

In almost half of the cases with false positive diagnosis then certain well defined characteristics could be distinguished which might be used to reduce the number of false positive diagnoses As to hypertension however, certain difficulties arise This condition is believed to have an atherogenic effect and to be an aetiological factor of IHD This means an overrepresentation of cases with symptoms of true IHD among those subjects who had hypertension with the result that it is difficult to decide which are true and which are false Neither is electrocardiography of differential diagnostic value because one of the requirements for the false pain is believed to be thickening of the left ventricular wall and in these conditions there are often cardiographic changes sometimes difficult to distinguish from those of myocardial damage Cases with valvular heart disease might however, offer a possibility of correction It is thus probable that the symptoms of IHD in younger subjects with valvular heart disease and an enlarged heart is less often due to true IHD

CHAPTER IX

Specificity, sensitivity and validity of typical and atypical clinical symptoms and certain electrocardiographic criteria

If a pilot study is to be used in the assessment of the magnitude of the sources of error of an epidemiological method, the series should be as representative as possible of the population in question. It seems difficult, however, to assess the errors of the methods for diagnosing IHD in a representative group because the possibilities of judging the true occurrence of IHD in such a series are limited. One must therefore resort to *post mortem* series which implies the introduction of various selective factors (cf. chapter I). These factors should be considered in the evaluation of the results.

The *specificity* is the ability of the criteria to exclude the possibility of the disease in a person not affected with it. The term thus designates the quotient between the number of healthy subjects screened out by the method as healthy in a given series and the true number of healthy subjects in the series (55, 56).

Sensitivity measures the ability of the criteria to detect all cases of a given disease in a given series and designates to the percentage of true positives detected (55, 56).

The sum of the two percentages sensitivity and specificity is a diagnostic score and gives supplementary information (55). If this sum is 100 it means that the criterion is the same as a random selection. If it is 200 it means that the method can identify all cases of a given disease without any missed diagnosis or overdiagnosis. Of these two numbers, 100 is an important limit at which the criterion should be regarded as valueless. It would, however, not be advisable to report only the diagnostic score or an analogous index (57) because one would then lose the valuable information obtainable from a comparison between the rate of specificity and sensitivity (55, 58).

Validity is more difficult to describe and it designates the extent to which the criterion measures the particular attribute it is intended to measure (3). In this case one can with reservation for that set forth above (page 78) regarding the limitation of the autopsy method postulate that IHD demonstrated post mortem is a valid sign of "true IHD". The percentage of post mortem diagnoses in a criterion group reflects an important aspect of the validity of the criterion.

The present analysis was confined

Table XXXII. Sensitivity, specificity and validity of certain criteria of IHD

	Total	Without IHD post mortem	With IHD post mortem	Specificity	Sensitivity	Agreement between clinical signs and post mortem findings
a. *Anamnesis*						
1. IHD anamnesis according to Rose, typical	203	[illegible]	137	91 %	46 %	65 %
Without IHD anamnesis according to Rose	865	711	154			
2. As under 1 and atypical anamnesis	268	99	169	8[illegible] %	[illegible]9 %	[illegible]3 %
Without IHD anamnesis	600	583	11			
b. *Previous ECG changes*						
1. Signs of infarction (according to Minnesota-code I 1—2, I 3 + V 3)	130	47	83	91 %	21 %	63 %
Without such changes	934	714	220			
2. As under 1 and left bundle branch block	220	70	150	89 %	41 %	9 %
Without such changes	844	691	153			
3. As under 2 and other changes in form of rhythm and conduction disorders (I 1—2, V 1—3, VI 1—4, VII 1—3, VIII 0—9, IX 1—3)	4[illegible]8	2[illegible]0	208	65 %	69 %	45 %
Without such changes	656	401	95			

to previous infarctions and angina pectoris, and therefore myocardial scars after infarction were the only criteria used for demonstrating IHD post mortem. The fact that the occurrence of coronary stenosis was not included as a criterion implies, as mentioned previously, only a minor source of error (page 48).

Table XXXII gives the specificity and sensitivity of the typical symptoms of IHD. The table also shows the variation of these qualities of the method when also atypical symptoms were accepted as signs of IHD. The lower half of the table gives an analysis of the various electrocardiographic signs of previous myocardial damage.

As previously mentioned, the data in the clinical records were sometimes not precise enough to allow satisfactory analysis. These uncertain cases were excluded in this analysis. This explains the differences between the number of cases studied for anam

nestic symptoms and those in which electrocardiograms were studied

Of those with typical symptoms of IHD 65 % had scars after infarction The criterion "typical symptoms", however catches only 46 % of all those with such scars in the present series 9 % of those without IHD postmortem have typical IHD symptoms (specificity 91 %) The values found for specificity and sensitivity give a diagnostic score of 137 for the typical symptoms If also atypical symptoms be accepted as signs of IHD it will probably increase the number of false positive diagnosis (4 % $\chi^2 =$ 5 2 fg 1 $p < 0\ 05$) At the same time it will increase the sensitivity considerably (13 % $\chi^2 = 10\ 0$ fg 1 $p <$ 0 01) This increases the diagnostic score to 146

As previously shown (page 21) the selective factors affecting this post mortem series probably implies an overrepresentation of cases with typical symptoms in relation to those with atypical symptoms This in turn suggests that the increase demonstrated in the sensitivity when also atypical cases were accepted would have been greater if a material had been available that was more representative of symptoms of IHD This would probably not have so great effect on the specificity

Electrocardiographic changes indicating local damage (according to definition) have a high specificity probably higher than that of the typical IHD symptoms ($\chi^2 = 4\ 57$ fg 1 $p < 0\ 05$) At the same time however its sensitivity is low Only one fourth of all cases with infarct scars were detected This means a low diagnostic score (118) If also those cases with left bundle branch block are included the specificity would be lower (6 %, $\chi^2 = 14\ 8$ fg 1 $p < 0\ 01$) This is compensated by a considerable increase in sensitivity (20 % $\chi^2 = 27\ 4$ fg 1, $p <$ 0 01) This would increase the diagnostic score to 132 If also a number of electrocardiographic changes such as arrhythmias and conduction disorders are also accepted the specificity will be reduced considerably Every third subject without post mortem signs of IHD would be judged as having IHD At the same time it would increase the sensitivity and 69 % of all cases in which post mortem showed IHD would be diagnosed Owing to this increase in sensitivity the diagnostic score of these criteria would be 134 Despite an equally high score this constellation of criteria cannot be recommended because of the poor balance between specificity and sensitivity This exemplifies the disadvantage of judging the criteria by the score only

The increase in sensitivity of the criterion left bundle branch block is strikingly large This is probably partly due to the high average age of the series As previously mentioned (page 36) certain qualities of this series presumably decrease the specificity of some electrocardiographic criteria, *i e* conduction disorders and arrhythmias

The criterion used at autopsy of IHD in this study was a myocardial scar larger than 1 cm in diameter As

mentioned this probably implies a source of error if IHD is to be understood only as a sequela after coronary atherosclerosis and thrombosis (page 78). This source of error causes systematic underestimation of the sensitivity of the criteria but should effect specificity only to a lesser extent. Since the main purpose of the present chapter *was to compare the sensitivity of* various criteria this systematic source of error is probably of minor importance. In all groups it will consist of a roughly equally large number of individuals with a false negative diagnosis.

In those investigations where these results are to be used as a basis for the elaboration of a method in population investigations the numerical value of sensitivity and specificity should be regarded as information of orienting nature. It is then important to *take account of deviating properties* of the post mortem series. The higher the age groups to be examined the more representative the afore mentioned results will probably be.

CHAPTER X

General discussion and conclusions

The question was whether atypical symptoms of IHD should be included as criteria of IHD in epidemiological studies. This question is important because such cases have proved fairly common (1, 2). The answer depends on how this would influence the balance between specificity and sensitivity of the anamnestic method. Since the atypical symptoms are not easy to distinguish from those of other diseases in the chest and upper abdomen it would probably result in an overdiagnosis of IHD, i.e. it would reduce the specificity of the method. In the choice of criteria it is of interest to know the size of this decrease of specificity in relation to the improvement of the method obtained by diagnosing an increased percentage of true IHD cases.

In the present investigation these problems were studied in a post mortem series in which anamnestic data and electrocardiograms were judged retrospectively. The study was a preparatory step in a planned prospective population investigation in Malmö. Symptoms of IHD *included in the definitions* recommended by WHO for epidemiological use were regarded as typical (see page 25). Symptoms not included in these definitions were regarded as atypical (see page 27).

Of the cases in the present investigation with symptoms of IHD the latter were atypical in 28 %. This percentage was, if anything, fictitiously low, for the material consisted mainly of a hospital series, i.e. a series in which an overrepresentation of cases with typical symptoms must be expected (page 21). This assumption is supported also by the higher frequency of myocardial scars in the subjects who had been autopsied at the department of forensic medicine and who had probably not been admitted to Malmö general hospital for previous myocardial infarction. They were therefore not included in the final series (see page 18).

The above assumption is supported further by the causes of death noted in the death certificates of those subjects who had died at home or at hospital for the chronic sick and were not autopsied and therefore not included in this series (see page 15).

Of greatest interest in a population study are the methods used for record

ing previous infarctions and angina pectoris One might *a priori* expect that cases with previous infarction are more difficult to detect than those with a recent infarction—this also proved to be the case in the present series Moreover, the frequency of false positive diagnosis was as expected somewhat higher among cases with atypical clinical pictures than in those in whom the disease had run a typical course (Table XX) *The acceptance of cases with atypical symptoms (Table XXXIV) produced a moderate decrease in the specificity and a marked increase in the sensitivity of the anamnestic method*

Electrocardiographic changes interpreted as signs of old myocardial damage (page 30) appears to be a valuable supplementary criterion and increases the validity of co-existing anamnestic symptoms Changes arguing for local damage have a high degree of specificity but such changes were found in only 21 % of the cases with myocardial scars That the sensitivity of this criterion is low has also been shown in population studies (35) The reason why the sensitivity is so low is probably partly that myocardial infarction does not always cause electrocardiographic changes of that type and secondly that such changes sometimes occur only in the acute phase and then regresses (60) It is also of interest to note that the electrocardiographic changes of this type in subjects who had denied anamnestic symptoms of IHD appear to have low validity (Table XXIV) No conclusive analysis of the criterion ST depression was performed because the series included many cases treated with digitalis and/or with disorders of the electrolyte balance

Earlier has been reported that specificity tends to vary inversely with the sensitivity (cf 55 56) This was the case also concerning the criteria of IHD that were studied The group of individuals decreasing the specificity was, however readily distinguished from the group that caused the decreased sensitivity It was therefore considered of interest to study these groups in detail to check whether it is possible to correct for the error of the method causing the decrease of the specificity and sensitivity

The source of error responsible for the decrease in *specificity* is the false positive diagnosis of IHD *i e* cases with IHD-like symptoms but without signs of IHD *post mortem* As mentioned above it is tempting to assume that other diseases of the chest and upper abdomen *e g* pneumonia chronic bronchitis pulmonary emboli peptic ulcer or gallstone can cause pain erroneously interpreted as IHD This assumption was checked in the cases of angina pectoris but no evidence for the above assumption could be found in this series On the other hand hypertension and valvular heart disease were more common in these cases than in sex- and age-matched controls These conditions were considered of pathogenetic importance because patients with these diseases had a higher average heart weight

than the controls and because an abnormally large heart muscle might perhaps be involved in the causation of the above mentioned symptoms In about half of the cases with a false positive diagnosis the heart weight was increased It is however questionable whether the presence of hypertension with enlargement of the heart can be used as a correction factor because hypertension is also believed to be an atherogenic factor This means that true IHD could be expected more common among individuals with hypertension and enlargement of the heart The exclusion of subjects with hypertension and enlargement of the heart from the group diagnosed as IHD would thus mean the exclusion of several cases with true IHD Evidence is however available that hypertension has a stronger coronary atherogenic effect in women than in men (22 53) This implies that a certain differentiation might be possible But before applying such a procedure the magnitude of the error introduced should be assessed Cases with valvular heart disease might however provide a possibility for correcting factors increasing the specificity of the method

The source of error reducing the *sensitivity* of the method is due to the false negative diagnosis of IHD *i e* cases in which myocardial scars were seen at autopsy in subjects without anamnestic symptoms of IHD or electrocardiographic signs of IHD

Various factors may be capable of masking symptoms of IHD It has thus been suggested that subjects with silent infarction consist mainly of old individuals with blunted perception of pain (28) or with hypertension (14) or of individuals who have had both myocardial infarction and vascular insult the latter disease making it difficult to obtain a satisfactory history of the heart symptoms (14) These factors however did not appear to be of importance in the present series but reservation should be made for the cases that were not included in the final evaluation because of inability to give a satisfactory history It appears difficult however to type the symptoms of IHD in such cases which can therefore not be classified as asymptomatic according to the used definition of the term

The group with silent infarction differed however in one respect from the symptomatic cases The atherosclerosis tended to be less severe and the most advanced type with occlusion and severe stenosis was significantly less common This was considered to argue for the silent infarctions possibly being caused to a certain extent by factors other than coronary atherosclerosis and related conditions These results suggest that if the purpose of the method is to detect sequelae after coronary atherosclerosis and related conditions the use of myocardial scars larger than 1 cm in diameter as criteria of IHD would mean a systematic underestimation of the sensitivity of the anamnestic methods and electrocardiographic criteria This methodological error however appears to be rather un

important in comparisons between different criteria in one and the same investigation.

It is also probable that several of these cases of infarction which might perhaps not have been of coronary atherosclerotic origin differed in size from other infarcts. They seem mostly to be within the range of 1—3 cm diameter (12, 20, 48). But further investigations are necessary to ascertain the limits and the error when these results are used for correction purposes.

In the present investigation cases with atypical clinical symptoms represented a considerable proportion of the cases with previous or chronic IHD. The anamnestic method seems to be able to distinguish them with only a moderately loss of specificity and at the same time with a substantial increase in sensitivity. In the application of these results the aim of the epidemiological method must be considered. If the epidemiological investigation is to be understood in its original limited sense comparison between populations in geographically different areas, special considerations must be taken into account. Of primary importance is then that the criteria correspond to the same underlying mechanism equally often in all areas studied, the validity of the criteria should be equal in the respective areas. This can cause problems concerning a certain part of the atypical cases. Some of these individuals experience their symptoms as "shortness of breath". This implies that the frequency of chronic bronchitis and emphysema will give rise to problems even if the discriminating distinction is applied that they should not have hyperpnoea. This problem is illustrated by an epidemiological investigation of miners and non-miners in South Wales (15). In that investigation it was stated that the history is difficult to interpret because "the discomfort of extreme breathlessness may be only too readily confused with the pain or discomfort of myocardial ischaemia". From the cited investigation it is clear that the diagnostic capacity of the anamnestic method is higher in the younger than in the older age groups. This applies in particular to miners in whom respiratory symptoms are common. It appears that in the cited investigation no attempt was made to subdivide the groups of anamnestic symptoms and to assess the relative diagnostic value of the individual symptoms, a procedure, which however, would have been difficult because they could only be compared with the electrocardiographic recordings.

It is against this background that one should consider the statement made by an expert group in WHO: "The epidemiologist will often be more interested in the repeatability of his observations than in their validity, if the two qualities cannot be combined. He will also as a rule be interested in the specificity rather than the sensitivity of the indices he uses, since he will be willing in general to discard atypical or debatable manifestations of a disease in order to obtain results comparable with those obtained by

other workers He will hope that the indices chosen will detect a rather constant fraction of the disease present in any population even though a fairly large number of probably cases may be discarded

The concession made in the introduction to the demands placed on validity may be questioned If it is not known what one is really measuring it would be difficult to interpret the findings

The results reported by Higgins (35) and those obtained in the present investigation however argue to a certain extent against the assumption that highly specific methods for the anamnestic diagnosis of IHD can give a fairly constant percentage of those with true IHD The exclusion of the cases with atypical symptoms probably implies the exclusion of a varying proportion of cases of coronary disease A possible alternative would be to state the number of cases with atypical symptoms per se and typical symptoms per se This would make it possible for every epidemiologist better to study the distribution of certain and probable respectively doubtful manifestations of IHD

These objections do not apply to the same extent to local electrocardiographic signs of myocardial damage provided the population is not selected from an area with a high incidence of trypanosomiasis for example On the other hand the frequency of ST depressions is influenced by the frequency of the use of digitalis medication—a factor of great importance in higher ages especially in women Since the indication for treatment with digitalis appear to vary from one area to another this is of importance in comparisons In some series attempts have been made to eliminate the influence of this factor by excluding subjects treated with digitalis But this introduces another source of error for many of these individuals probably have IHD which is a common cause of cardiac incompensation in high ages Exclusion of these individuals would therefore imply the exclusion of a group with a high frequency of IHD and this would influence the results in the remaining group

The term epidemiological however is no longer limited to comparisons between different geographical areas but also comprises comparisons between different groups in the same population e g social groups' occupational groups and groups selected according to dietary and smoking habits and symptoms of disease (25) In such intra and intergroup studies it is often of value to distinguish one group with a high frequency of the disease and another group without symptoms or signs of the disease This however increases the requirement of knowledge of the properties of the criteria

It is clear from the above that no well defined general method can be recommended In the planning of an epidemiological study the methods used must be devised with the due consideration to the composition of the material and the purpose of the investigation Besides evaluation of the validity of the method the ratio

between the specificity and sensitivity must also be considered and then the results presented here may serve as a basis

In the choice of method internationally recommended criteria should, of course be preferred In most cases various criteria can be used simultaneously to distinguish several groups and thereby provide the possibilities of comparisons within groups in the same series and with other series

Summary

Recent investigations have shown that atypical symptoms of ischaemic heart disease (IHD) are fairly common. This gives rise to problems in the evaluation of the disease in population studies. This retrospective investigation was undertaken to elucidate questions bearing on these problems.

The study is a preparatory link in a planned prospective population study in Malmö, a town of 250,000 inhabitants. Malmö offers a favourable opportunity for a combined clinico-pathological study of this type because it is a one hospital town.

Originally the series consisted of all individuals who had died during one and a half year in Malmö (2 880 individuals). 1 435 of these cases had both autopsies and clinical records. In about 1 050 the clinical records fulfilled the requirements for the intended analysis.

The exclusion of some groups might influence the representativity regarding symptoms of IHD, and this bias was analysed as was the validity of the data from the hospital records.

Anamnestic data, electrocardiographic recordings and the results of laboratory studies were obtained from the hospital records and compared with the results of a careful autopsy at which special attention was given to the cardiovascular system.

Of the same 1 000 individuals 286 had old myocardial scars. Of these 40 % denied ever having had heart symptoms. Of those who reported heart symptoms 74 % had had typical symptoms and 26 % had had atypical symtoms. The anamnestic criteria defined by Rose and recommended by WHO were called typical (page 25). Pain in other regions and other precipitating factors were accepted as atypical symptoms (page 27).

When the cases with atypical symptoms were assigned to the group with typical symptoms to form a total group with anamnestic symptoms of IHD the sensitivity of the anamnestic method increased and then 59 % instead of 46 % of all cases with post mortem signs of IHD were diagnosed. At the same time a probable decrease of the specificity was noted from 91 to 87 %, which means that 13 % instead of 9 % of those who did not show evidence of IHD *post mortem* had been judged as having IHD. Thus

when the cases with atypical symptoms were excluded it meant a considerable decrease in the sensitivity of the anamnestic method, while when these symptoms were accepted as signs of IHD it implied some decrease of the specificity

The value of anamnestic symptoms in relation to that of electrocardiographic changes is discussed In the present series the anamnestic method appeared to be valuable as a diagnostic procedure, while changes in the resting electrocardiogram (classified according to the criteria of the Minnesota code) increase the value of an anamnestic symptom but are of poor validity in the absence of anamnestic symptoms of IHD The anamnestic symptoms and electrocardiographic changes appeared to be more specific throughout in males than in females

Of the specificity reducing group of the cases with angina pectoris i e those with angina pectoris like symptoms but in whom post mortem examination revealed no signs of IHD about half had valvular heart disease and hypertension and the results suggest that these conditions might be the cause of the angina pectoris like symptoms No evidence was produced of other diseases in the thoracic cavity or upper abdomen such as pneumonia *bronchitis pulmonary embolism or* peptic ulcer simulating IHD in the group with angina pectoris

As to the sensitivity decreasing group, *i e* cases without IHD in their history or electrocardiographic changes but with myocardial scars seen post mortem (silent infarction) previously suggested masking factors such as high age cerebral insult or hypertension were not found to be of importance in this series The results suggest that coronary atherosclerosis and related factors are less advanced in these cases with silent infarction than in those with symptoms and autopsy signs of infarction Possible aetiologic factors other than atherosclerosis for some of these cases are discussed

The role played by atypical symptoms in comparisons between different geographical areas is discussed

In the choice of method and in the subdivision of the material for comparisons within and between groups with different IHD symptoms the relation between specificity and sensitivity of the criteria and the reproducibility must be decided upon with due regard to the problem to be studied In this choice internationally recommended methods should be given preference but special criteria should sometimes be adopted The results of this retrospective study provide a basis for this choice in the *planned prospective study in Malmö*

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SUPPLEMENTUM 475

MITRAL INCOMPETENCE AS A COMPLICATION OF ACUTE MYOCARDIAL INFARCTION

BY

JUHANI HEIKKILA

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To the memory of my father

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 475

FROM THE FIRST MEDICAL CLINIC, UNIVERSITY OF HELSINKI,
AND FROM THE WIHURI RESEARCH INSTITUTE,
HELSINKI, FINLAND

MITRAL INCOMPETENCE AS A COMPLICATION OF ACUTE MYOCARDIAL INFARCTION

BY

JUHANI HEIKKILA

ACADEMIC DISSERTATION

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Helsinki, March 1967

Juhani Heikkilä

INTRODUCTION

"There is perhaps in the animal frame no more beautiful example of the adaptation of structure to the function to be accomplished than is afforded by the auriculo ventricular valves The insertion of the cords into the fleshy columns instead of directly into the muscular walls, is apparently not to give greater power of resistance to the pressure of the blood during the systole, but to furnish a means of shortening the attachments of the curtains when, with the contraction of the ventricle the walls are more closely approximated Were it not for this arrangement the free fold of the mitral, for instance, would fall back towards the auricle during the systole and the two curtains not being properly adjusted the blood would flow into the auricular cavity By the action of the muscular columns, however, the cords are drawn upon as the parietes of the ventricle approximate, and the curtains are kept in apposition and tightly stretched across the aperture so as effectually to close it"

This penetrating description of the function of the papillary muscles by Thomas B Peacock dates from 1865 The correctness of the theory has later been confirmed by means of modern physiological research

The leakproof closing of the mitral valve during systole depends on the flawless structure and function not only of the valve leaflets themselves but also of all the other anatomical structures involved — the supporting annulus of the mitral orifice, the chordae tendinae the papillary muscles, the left ventricular wall and the atrial myocardium

The initial phase of the closing mechanism begins during the atrial contraction at the end of diastole This augments the filling of the ventricle With the intraventricular pressure rising the chordae tighten simultaneously as the distance between the mitral annulus and the base of the papillary muscles increases Thus the cusps of the mitral valve are prepared for closing and in fact, they close loosely at this final diastolic stage Regarded as factors contributing to the closing process are the abrupt arresting of the diastolic blood flow and the flow accelerated by the atrial systole together with the eddies produced by it The ventricular systole begins with the isometric contraction of the papillary muscles This anchors the valve leaflets against the systolic rise of the intraventricular pressure and tightly occludes the mitral orifice During the ejection phase as the volume of the ventricle decreases, the active contraction of the papillary muscles apparently takes up the ballooning thus induced on the atrial side The considerable active diminishing of the circumference of the mitral orifice as a result of the contraction of the ventricle further ensures the strength of the competence of the valve

Because the closing mechanism of the

mitral valve is both structurally and functionally highly complicated, it is vulnerable to numerous pathological processes. As a consequence of any inadequate functioning of the valve and its supporting structures, a mitral incompetence develops.

Anatomical conditions that frequently cause extensive damage to the mitral valve have been long known. The most important of them are rheumatic and bacterial inflammations causing deformation and substance loss of the valve cusps as well as shrinkage or breakage of the chordae. The valve structure may have various congenital defects. The role played by the active contraction of the mitral annulus in the closing mechanism may be eliminated by advanced calcification. If a papillary muscle is ruptured, most commonly in connection with a chest injury or in myocardial infarction, the consequence is a sudden catastrophe in the functioning of the heart, most often fatal.

In contrast to the organic injuries to the valve structure easily observed at autopsy, increasing attention has been paid to the mitral incompetence resulting from a structurally unchanged but functionally impaired mitral valve. And indeed, it is only since the development of appropriate instruments that it has become possible to investigate the phenomen closely.

Myocardial infarction may produce sudden and often severe difficulties in the ability of the heart to function as a pump to maintain circulation. Evidence of acute mitral incompetence caused by various ischemic mechanisms, has emerged as one possible factor impairing the efficient pumping action of the ischemic heart. Mitral incompetence associated with ischemic heart disease might be either structural or functional — or a combination of these two mechanisms.

The occurrence of mitral incompetence as a complication of myocardial infarction has been long known. The prevalence of this complication and its clinical features do not, however, emerge clearly from the previous studies dealing with the subject. The character of hemodynamic disorders also remains to be elucidated. In the present study, an effort has been made to clarify, by means of a prospective investigation, the occurrence and the clinicopathologic features of mitral incompetence as a consequence of acute myocardial infarction.

A preliminary report of the results of this study has been published (1967).

SURVEY OF THE LITERATURE

CLINICAL OBSERVATIONS

SYSTOLIC MURMUR ASSOCIATED WITH MAJOR COMPLICATIONS OF MYOCARDIAL INFARCTION

The appearance of a loud systolic murmur during the course of acute myocardial infarction is fairly uncommon, and it has been described as generally being associated with major complications of infarction with the exception of pericarditis These sudden and often fatal complications causing systolic murmurs are perforation of the interventricular septum, rupture of the papillary muscle, rupture of the heart and aneurysm of the heart

Perforation of the interventricular septum This has been reported in over 200 cases (Sager 1934 Bond 1953, Malone and Parkes 1955, Sanders *et al* 1956) Brunn (1923) made the first clinical diagnosis which was confirmed at autopsy Usually on the 3rd or 4th day, in connection with an infarct extending to the area of the septum a loud pansystolic murmur appears which is typical of the ventricular septal defects This is accompanied in some 50 % of the cases by a systolic thrill Simultaneously, the patient s general condition worsens rapidly after a sudden, severe right sided heart failure Pulmonary plethora *in the chest radiograph can help* diagnosis More than half the patients die within a week of heart failure, and only about a tenth of them survive two months

Rupture of a papillary muscle, or rupture of the chordae tendinae — which extremely seldom occurs as a complication of myocardial infarction — also immediately brings on a severe heart failure, almost invariably left sided The murmur generally appears 2 to 4 days after the onset of the infarction, and it is typical of a substantial mitral incompetence being pansystolic apical, and radiating toward the axilla but also, deceptively, often toward the aortic area (Craddock and Mahe 1953 Sanders *et al* 1957) Usually there is no thrill The posterior papillary muscle of the left ventricle ruptures considerably more often than the anterior one Sudden mitral incompetence terminates fatally in over half the patients within the first 24 hours Approximately 90 cases of ruptured papillary muscles have been described (Cederqvist and Soderstrom 1964) The first antemortem diagnosis was made by Davison in 1947

Rupture of the heart causes some 10 % of the hospital deaths from acute myocardial infarction (Gans 1951, Nesvadba 1955 Maher *et al* 1956 Griffith *et al* 1961) Rupture of the heart most commonly occurs between the 2nd and 4th day after onset, and only very seldom after a period of three weeks A myocardial rupture is almost without exception a complication that terminates fatally within a few minutes or at most, hours and *therefore can only rarely be diagnosed* clinically The symptoms include signs of severe tamponation of the heart, in some case reports a systolic murmur has been described (Reznikoff 1922 Massey and Drake 1948, Bishop and Logue 1950, Nuzum 1950)

Aneurysm of the heart as a clear anatomical finding after infarction appears in some

20 % of the cases at autopsy (Schlichter *et al.* 1954, Abrams *et al.* 1963), whereas dynamic aneurysm, consisting of a systolic bulging of the ventricular wall after weakening by ischemic damage, can be observed and registered in up to 100 % of the patients during the acute stage of infarction, with permanent signs remaining in more than half of the patients (Prinzmetal *et al.* 1949, Gregg 1950, Schwedel *et al.* 1950, Sampson *et al.* 1956, Suh and Eddleman 1959, Davie *et al.* 1962, Kurtzman and Lofstrom 1963, Rörvik 1963, Schweitzer *et al.* 1965).

A clinical consequence is often a distinct heart failure. Pathological pulsation of the heart is reported as having been observed clinically in 10 % of the patients (Vakil 1955, Logue and Hurst 1966 b); moreover the diagnosis is supported by permanently elevated ST segments in the electrocardiogram, deformation of the heart shadow and paradoxical motion in radiological examination. In connection with an aneurysm it is possible at times to hear a varying systolic or diastolic murmur (Scherf and Brooks 1949, Slapak 1952, Riederer and Themel 1955, Vakil 1955, Plotz 1957). The murmur has most often been attributed to mitral incompetence but sometimes to the flow of blood in and out of the aneurysm, or to pericardial friction.

INCIDENCE OF SYSTOLIC MURMUR IN MYOCARDIAL INFARCTION

With the exception of the afore mentioned severe complications of myocardial infarction, the literature contains very scant references to the occurrence of a systolic murmur in clinical studies of myocardial infarction. Bean (193[illegible]) mentions a systolic murmur as having been detected in [illegible] of 170 patients. Jacobs (19[illegible]) observed that a systolic murmur was present in 11 patients out of 84 examined. M[illegible] and R[illegible]enberg (19[illegible]) found it in [illegible] 44 patients. Yater *et al.* (194[illegible]) [illegible] patients a systolic murmur, which in 32 patients was heard over the mitral area. All the murmurs were transient, none were considered characteristic of any definite valvular lesion. In these studies, except for its frequency, no detailed description of the murmur or a possible explanation of its origin has generally been given. Accordingly, no conclusion concerning the origin, whether aortic or mitral, can be drawn.

According to the observations of Schumert *et al.* (1960), the systolic murmur of aortic sclerosis established before the onset of infarction is apt to disappear completely during the hypotension or shock stage of the initial phase of infarction as the cardiac output decreases. This ejection type murmur can be heard again as the circulatory conditions gradually return to normal and the stroke volume rises.

The pansystolic apical murmur often detected after reinfarctions or after the development of congestive heart failure has customarily been explained as a relative mitral incompetence resulting from dilatation of the failing left ventricle (Laubry and Soulie 1950, Friedberg 1966). Laubry and Soulié (1950) explained this mechanism by pointing out that the gallop rhythm frequently accompanied the appearance and disappearance of the apical murmur of relative mitral incompetence. This murmur has frequently been mentioned but its incidence has not been discussed. The systolic murmur of a mitral incompetence ascribed to ventricular dilatation occurring in conjunction with infarction was already described by Vaquez (1921), who reported it to be meso- or protosystolic, seldom pansystolic, apical, unradiating, and fairly faint.

ISCHEMIC PAPILLARY MUSCLE DYSFUNCTION AS A CAUSE OF MITRAL INCOMPETENCE

Reports on clinical series of mitral incompetence without papillary muscle rupture in association with ischemic heart disease are few in number and often some-

what cursory (Grotel 1940 Nezlin and Shamesova 1951, Froment *et al* 1955, Phillips *et al* 1963 a, 1963 b Orlando *et al* 1964, Segal and Likoff 1964 Bashour 1965, Chiesa *et al* 1965, Raftery *et al* 1966, Soulie *et al* 1966)

In addition to these series mostly consisting of materials studied in retrospect a few case reports have been published (Soulie and Gerbeaux 1939, Hope and Askey 1952 Luisada and Szatkowski 1960 Burch *et al* 1963 Mazzitello 1964, Clinicopathological conference at the Postgraduate Medical School of London 1965 Holloway *et al* 1965 Tavel *et al* 1965, Fluck *et al* 1966) So far no systematic prospective studies on the occurrence, clinical characteristics and subsequent course of mitral incompetence developing in association with acute myocardial infarction have been presented

Several authors (Bristowe 1861, Peacock 1865, Gee 1870, Heveling 1871 Chabardes 1878 Gibson 1898) in the 19th century published occasional case reports in which mechanical failure or "fatty degeneration of papillary muscles was shown to be the cause of clinically observed mitral incompetence In those days of course the concept of myocardial infarction had not yet been clarified beyond the stage of ' myomalacia

Castex (1931, 1933) presented eight patients with a faint ' merosystolic murmur (filling part of the systole), in connection with myocardial infarction as a distinct clinical entity The murmur was mesosystolic or mesotelesystolic in patients with damage to the posterior wall of the heart and protosystolic in apical infarctions Castex presented his observations in contradiction to previous contentions by Barie (1912) and Potain that a merosystolic murmur was invariably non organic and usually extracardial Vaquez (1921) also stated that the question was one of a possible functional mitral incompetence only and not an organic impairment Castex (1932) expounded the mechanism of the murmur however, in the light of the vortices autoctonos theory of Bondi (1927, 1928 a, b), according to which the discrepancy between the vigorous contraction of the healty infundibular part of the myocardium and the poor motility of the damaged often dilated region of the infarction, induced eddies in the cavity of the left ventricle, thereby giving rise to the murmur From Castex's clinic a few case reports interpreted according to his way of thinking also appeared (diCio and Battro 1933, Lorenzo *et al* 1934 Lorenzo and Boto 1936)

However in the light of later research it can be assumed that the cause of the murmur in at least some of the patients described by Castex was a mitral valvular leak caused by an ischemic functional disorder of a papillary muscle For example, in Fig 2 (Castex 1933), apart from the extensive scarring of the left ventricle the papillary muscle appears to be scarred and considerably shrunk

It was Bosco (1935) who was the first to contend, on the strength of his illuminating coronary arterioradiographic investigations that the mechanism of the merosystolic murmur occurring in connection with infarction was a functional disorder of the papillary muscles resulting from their deficient blood supply No clinical series was described chief stress being laid on the pathologic anatomic investigations relating to the blood vessels of the papillary muscles

Subsequent to Castex s clinical study, which may have touched upon the category of mitral incompetence and Bosco s unequivocal thesis, the possibility of mitral incompetence resulting from ischemic damage to the supporting structures of the mitral valve has not been seriously considered

Incidence of murmur

The incidence of mitral incompetence as a consequence of myocardial infarction has been treated in only two stud

ies based on a large material. The Russian Grotel (1940), in his work on acute myocardial infarction, mentions an incidence of 50 %, which was astonishingly high in view of the syndrome's being fairly unknown before then. However, Froment *et al.* (1955) also observed the systolic murmur of a mitral incompetence in 48 of 189 patients with myocardial infarction and drew attention to the possibility of [redacted] mitral incompetence occurring probably because of functional impairment of a papillary muscle in a normal sized heart and without heart failure. This observation proved contrary to the previously common assumption that relative mitral incompetence was secondary to dilatation of the heart and its annulus caused by heart failure. Unfortunately, the material of Froment *et al.* has not been analyzed further than is shown in Table 1.

Hochrein (1937) remarked that Castex's observation concerning the localizing value of the apical systolic murmur attendant upon infarction appeared in the light of his own investigations to possess „keine allgemeine Gultigkeit".

Characteristics of the murmur

The ejection type or "diamond shaped" systolic murmur, with a delayed onset only after a phase of the isovolumetric contraction confirmed phonocardiographically, was described as a characteristic finding in ischemic papillary muscle dysfunction in 13 patients by Burch *et al.* (1963) and Phillips *et al.* (1963 a). Another typical feature shared by all these patients was electrocardiographic changes attributed to infarction of the anterolateral papillary muscle of the left ventricle. Together they composed an electrocardiographic-auscultatory syndrome easily recognized clinically. The ejection type murmur was fairly loud, apical and apt to radiate toward the axilla, though in four patients it radiated toward the aortic area. The origin of this murmur in the mitral valve was demonstrated by a decrease in its intensity in the amyl nitrite test.

An ejection type but late systolic and more crescendo-shaped murmur is also mentioned by Segal and Likoff (1964) as having been observed in 20 patients with anterior myocardial infarction but without aneurysm. All 3 patients described by Tavel *et al.* (1965) also had a late systolic murmur. In one of them [redacted] began with a non ejection click. But although the authors connected the murmur of two of the patients with definite coronary heart disease, the case reports reveal that in both the murmur had appeared several years before the symptomatic chest pains, thus throwing some doubt on the connection.

According to the majority of writers, most of the murmurs appearing in association with myocardial infarction would seem, however, to be pansystolic (Grotel 1940, Nezlin and Shamesova 1951, Froment *et al.* 1955, Friedland and Fishleder 1960, Orlando *et al.* 1964, Raftery *et al.* 1966). The pansystolic murmur [redacted] apt to exhibit various types in phonocardio-

Table 1. *Occurrence of mitral incompetence after myocardial infarction in material of Froment et al. (1955)*

	Number of infarctions	Number of patients with mitral incompetence	Mitral incompetence with dilatation of the heart	Mitral incompetence without dilatation of the heart
Anterior infarction	113	15 (13 %)	14	1 (0.8 %)
Lateral infarction	76	[redacted] (43 %)	12	21 (27 %)

graphy and even to vary in the same patient (Bashour 1965) In 5 patients with myocardial infarction who belonged to a series of 23 patients with acute subval var mitral incompetence (Raftery *et al* 1966), the murmur was invariably pan systolic with a mid systolic cresendo

The intensity of the murmur varies and has generally been reported to be fairly loud or sometimes as rather faint (Grotel 1940)

Grotel (1940) and Phillips *et al* (1963 a) further mention having detected late systolic apical murmurs which could be heard transiently only during attacks of angina pectoris Similarly, Bolechowski and Poplewski (1964) described a loud, musical pansystolic murmur during an gina pectoris that disappeared when the pain subsided Post mortem examination disclosed several post infarction scars in the posterior wall of the left ventricle According to the observations of Grotel (1940) and Nezlin and Shamesova (1951) the murmur gradually increases in inten sity during the days immediately following its appearence and does not suddenly become loud as in conditions of rupture of a papillary muscle

There are only scanty reports on the time of onset of murmur as most of the studies made have been retrospective in character Among Castex's (1931, 1933) patients, the merosystolic murmur appeared on the 4th or 5th day In 8 patients out of 10 with severe myocardial infarction involving papillary muscles and terminat ing fatally, the murmur described by Nezlin and Shamesova (1951) appeared between the 3rd and 7th day after the onset of the disease According to Grotel (1940), the murmur is even apt to develop within a few hours after the symptoms of infarction have been noticed and gener ally within 24 to 28 hours

The murmur has generally been de scribed as permanent but it can also be transient (Castex 1931, 1933, Grotel 1940) and as pointed out in the foregoing merely brought on by angina pectoris The location has nearly always been described as apical, with a tendency for transmission to the axilla, but occasionally radiation toward the aortic area (Grotel 1940 Nezlin and Shamesova 1951, Phil lips *et al* 1963 a, Raftery *et al* 1966)

Clinical significance

The significance of a mitral valvular leakage as a result of myocardial infarct ion varies Froment *et al* (1955) empha size the possibility of a lack of heart failure Similarly the patients studied by Burch *et al* (1963) and Phillips *et al* (1963 a), who for the most part had only a subtransmural myocardial damage were in fairly good condition Neither did the ejection type or late systolic murmur discussed by Holloway *et al* (1965) and Tavel *et al* (1965) have any relation to heart failure

On the other hand Bashour (1965) stresses the susceptibility of patients to severe heart failure, while Nezlin and Shamesova (1951) view this complication as exceedingly grave, leading almost in evitably to fatal heart failure Pulmonary edema was the initial symptom in all the patients described by Raftery *et al* (1966) The possibility of bacterial endo carditis complicating mitral incompetence even when caused by myocardial infarct ion was suggested by Bashour (1965)

Radiological findings

Radiological examination of the chest may reveal the size of the heart to be normal or conspicuously enlarged (Grotel 1940, Froment *et al* 1955, Phillips *et al* 1963 a Raftery *et al* 1966) Grotel (1940) and Froment *et al* (1955) specifically emphasize normal heart size as pointing to a malfunction of the papillary muscle and as evidence against the mechanism of mitral annular dilatation Raftery *et al* (1966) note that the left atrium remains normal in size or enlarges only negligibly in acute mitral incompetence, despite

very severe hemodynamic disorders

Electrocardiographic changes

In most studies the occurrence of mitral incompetence has been maintained to be more common in cases of inferior or posterior infarction than in those of anterior infarction (Castex 1933, Grotel 1940 Nezlin and Shamesova 1951 Froment *et al* 1955, Orlando *et al* 1964 Bashour 1965 Raftery *et al* 1966 Soulie *et al* 1966) Burch *et al* (1963) Phillips *et al* 1963 a b) and Segal and Likoff (1964) deal with anterior myocardial infarction only Dolgoplosk (1956) found electrocardiographic signs of right ventricular overloading to be common in connection with this syndrome

Phillips *et al* 1963 b) maintain that infarction of the anterolateral papillary muscle of the left ventricle can be reliably diagnosed from an electrocardiogram. The changes include a slight or a marked depression of junction J and a concave or convex upward deformity in the ST segment which may or may not be accompanied by a negative T wave Furthermore in the majority of the tracings changes in the TU segment or the U wave were observed In both patients of Holloway *et al* (1965) the type II (Phillips *et al* 1963 b) changes of the ST-T deflection were present. U wave changes had already been regarded previously as specific signs of papillary muscle damage (Furbetta *et al* 1956)

However, it is hard to understand how the changes described by Phillips *et al* (1963 b) could be specifically distinguished from the electrocardiographic changes present in subendocardial damage, with or without involvement of the papillary muscles, to the wall of the left ventricle (Cook *et al* 1958) The ischemic changes found in the majority of patients studied by Phillips *et al* (1963 a, b) were limited and stationary, non transmural and without Q waves In acute transmural infarction the afore mentioned electrocardiographic signs would not seem to be operable on account of the extensive electric changes of injury and ischemia

AUTOPSY FINDINGS AND MECHANISM OF MITRAL INCOMPETENCE

The earliest thesis on the part played by malfunction of the papillary muscles in mitral incompetence without papillary muscle rupture in connection with myocardial infarction is as already mentioned that comprising the conclusions arrived at by Bosco in 1935 on the basis of the coronary radiographic investigation on the blood supply of the papillary muscles Soulie and Gerbeaux (1934) also mention this possibility of functional impairment of the papillary muscles although they finally regard the eddies produced in the blood stream or the mechanical hindrance to coapting of mitral leaflets caused by the large mural thrombus found at autopsy as the cause of the murmur

Nezlin and Shamesova (1951) have thoroughly elucidated the pathologic-anatomical changes involved in myocardial infarction complicated by an acute mitral incompetence. Papillary muscle necrosis was observed in 20 % of the cases They pointed out the greater vulnerability of the posterior papillary muscle and discuss this observation with reference to the

anatomy of the coronary blood vessels The same conclusion was reached by Orlando *et al* (1964) and Bashour (1965) Like most of the case reports, these studies generally stress the importance of necrosis or of ischemia of the papillary muscle, usually posterior as a mechanism of a mitral incompetence as contrasted with the previously held view of the relative mitral incompetence secondary to dilatation of the heart

Burch *et al* (1963) and Bashour (1965) present a further mechanism in which even a healthy papillary muscle attached to an infarcted and aneurysmatic area of the left ventricle may result in inadequate closure of the mitral valve This is due to the paradoxical outward bulge that occurs during systole pulling the site of attachment of the papillary muscle away from the mitral ring In the gradual process of dilatation undergone by the heart the papillary muscles are capable of hypertrophying and, by lengthening adapting themselves to the increased distance from the mitral ring without leakage occurring In acute dilatation such a compensating mechanism is obviously not possible

In their series of 4 patients Soulie *et al* 1966) emphasize the importance of the fibrosis and shrinkage occurring as the end result of the ischemic destruction of the papillary muscle in producing severe permanent mitral incompetence as do Phillips *et al* (1963 a), Bashour (1965), and Marton (1966)

Recently Moller *et al* (1966) described mitral incompetence associated with congenital aortic stenosis The mechanism of mitral incompetence was considered to be extensive infarct and fibrotic changes in the papillary muscles and the subendocardial myocardium caused by inadequate coronary circulation The same mechanism of mitral incompetence traceable to the damaged papillary muscles can also be observed when the left coronary artery arises anomalously from the pulmonary artery (Noren *et al* 1964)

From the viewpoint of the pathologist, retromitral infarcts often leave no harmful effects (Bargmann and Doerr 1963) Still if the infarct extends to the area of the papillary muscles and the heart becomes slightly aneurysmatic, the consequent slackening of the papillary muscle and the posterior wall of the left ventricle is attended by extra valvular mitral incompetence and enlargement of the left atrium

In an autopsy study of 41 patients who had died of myocardial infarction Arkhangelskij (1959) found necrotic damage to the papillary muscles of the left ventricle in over 50 % Both papillary muscles were involved in 25 % of the patients regardless of the location of the infarct Total necrosis and sclerosis were commoner in the posterior papillary muscle In the papillary muscles of control subjects who had died without infarction from hypertension or atherosclerosis changes were not always found and if they were they consisted of slight sclerotic foci Oeser (1954) observed an involvement of the posterior papillary muscle in 20 % of patients who had died of myocardial infarction

DePasquale and Burch (1966) found gross scars in one or both papillary muscles in 21 % of 420 consecutive autopsies in men Acute infarction of the papillary muscles was present in 4 % The authors suggest associating these findings with common apical systolic murmurs in elderly subjects Careful pathologic anatomical studies made by Fulton (1965) revealed that the papillary muscles which must be classified as subendocardial tissue, are sensitive to ischemic changes

By contrast, Schoenmackers (1965) could not find clear association between papillary muscle infarcts and acute mitral incompetence

HEMODYNAMIC STUDIES

Hemodynamic changes investigated by cardiac catheterization in patients with mitral incompetence after myocardial infarction (excluding papillary muscle ruptures) had been reported in only 4 patients before the present study was started (Luisada and Szatkowski 1960 Mazzitello 1964 Holloway *et al* 1965) As awareness of this complication has rapidly grown during the past two years, further observations have been made with respect to changes in hemodynamic conditions (Bashour 1965 Raftery *et al* 1966 Soulie *et al* 1966 Fluck *et al* 1966) A total of 10 patients have figured in reports published on catheterization studies.

The investigations prove the existence of hemodynamically significant mitral incompetence The *v* wave of the left atrium was generally over 40 mm Hg (15–65) and the end-diastolic pressure of the left ventricle had risen

The left ventricular cine angiocardiograms revealed a substantial regurgitation into the left atrium from the site of one mitral cusp or the other and frequently a systolic prolapse of the valve into the left atrium (Mazzitello 1964 Holloway *et al* 1965 Bashour 1965 Soulie *et al* 1965 Raftery *et al* 1966 Fluck *et al* 1966) One patient was successfully operated on by inserting an artificial mitral valve prosthesis when the clinical course and hemodynamic studies revealed intractable mitral regurgitation after acute myocardial infarction (Fluck *et al* 1966)

EXPERIMENTAL STUDIES

Myocardial ischemia The effects on cardiac function of experimental serial ligation of branches of the circumflex and of the anterior descending branch of the left coronary artery in 17 dogs were studied by Bailas 1965) Pressures were measured in the heart chambers, and left ventricular angiography was performed on three of the animals In 9 53 % of the 17 animals with evidence of severe myocardial injury and left ventricular failure, mitral regurgitation of a significant degree was observed. A systolic thrill was palpable in all nine animals Mitral incompetence was explained by the failure of the ischemic papillary muscles to contract and their inappropriate position when left ventricular failure produced acute ventricular dilatation.

Papillary muscle damage Hider *et al* 1965 1966 studied the effects on dogs of an ethanol injection in the papillary muscles. Control dogs were injected in the lateral wall of the left ventricle. Of the 11 experimental animals 3 showed immediate mitral incompetence leading to pulmonary edema and two of them died After three months the combined early and late incidence of mitral incompetence was 7 out of the 11 dogs (64 %) in the experimental group and 1 (atriotomy scar deforming the anterior mitral leaflet) out of 10 in the control group Mitral regurgitation was estimated by left atrial pressure curves and by an Evans blue injection in the left ventricle and withdrawal from the left atrium. A tendency was further observed in the experimental group towards less efficient isometric contraction of the left ventricle. In the view of the authors the results supported the predominant role of the papillary muscles during the isometric rather than the ejection phase of the ventricular systole.

Studies of Mantini 1967) also showed in connection with mitral valve replace

ment surgery the importance of papillary muscles for the contractility of the left ventricle

To sum up there are few studies on mitral incompetence as a consequence of acute myocardial infarction and most of them deal with small series of patients studied retrospectively In the light of the results presented both the clinical features and the character of the murmur itself are inconsistent and variable On the other hand there are clear indications that the functional impairment of the papillary muscles manifesting in various ways is the critical factor in the appearance of mitral incompetence. Pathologic anatomical and experimental investigations suggest that the clinical frequency of mitral incompetence should be higher than has usually been reported Few hemodynamic studies have been published and they deal with selected patients only In them, mitral incompetence has on the whole been found to be of a significant degree

OBJECT OF THE PRESENT INVESTIGATION

The object of the present investigation was to study the clinical features and the significance of mitral incompetence as a complication of acute myocardial infarction. The main emphasis was given to clarifying the following problems.

— The prevalance of mitral incompetence as a complication of acute myocardial infarction.

— The time of onset and the characteristics of the murmur of the mitral incompetence.

— The possible differences between anterior and posterior infarction with respect to the occurrence and character of the mitral systolic murmur.

— The clinical features in patients with myocardial infarction complicated by an acute mitral incompetence as compared with those in patients without mitral incompetence during the acute phase of the disease.

— The underlying mechanism of a mitral incompetence.

— The severity of the hemodynamic changes in mitral incompetence resulting from myocardial infarction as revealed by cardiac catheterization studies during the convalescence stage.

— The permanence of a mitral systolic murmur and the long-term effects of mitral incompetence in patients with myocardial infarction.

METHODS

A DEFINITIONS AND EVALUATION OF CLINICAL FINDINGS

The result of a clinical examination depends a great deal on how accurately a symptom or sign is defined and on this definition being maintained for otherwise the information yielded by the examination would disintegrate in uncertainty and the significance of the result would remain difficult to evaluate On the other hand taking into account only such signs that very conspicuously deviate from the normal often gives a one-sided and erroneous picture of the matter under examination In the following the most important clinical concepts and the criteria applied to them will be defined and explained

Myocardial infarction is defined as an area of necrosis in the myocardium resulting from cessation of the coronary blood supply or of its curtailment below the myocardial demand and is associated with a distinctive clinical picture

The clinical picture is characterized by a severe prolonged substernal chest pain often radiating to the shoulders and hands independent of physical exertion It tends to be attended by left ventricular failure and shock marked electrocardiographic changes rising body temperature elevated erythrocyte sedimentation rate and leucocyte count and an increase in certain cell enzymes

In the present study the infarction diagnosis was determined if the clinical picture was consistent with myocardial infarction and/or a pattern of electrocardiographic changes in myocardial infarction were observed either as pathologic Q waves together with ST-segment and T wave changes indicative of injury or only fresh distinct ST-segment and T wave changes indicative of subtransmural infarction Laboratory tests were used to support the diagnosis if the erythrocyte sedimentation rate rose by 10 mm an hour or more the white blood cell count over 9 000 mm³ and the serum GOT over 40 units (Wróblewski 1959) which was the upper limit of the laboratory In most patients the serum LDH levels were likewise determined However the clinical picture in conjunction with unequivocal electrocardiographic changes was regarded as justifying a diagnosis although the laboratory tests did not change to a pathologic degree Insofar as the electrocardiographic changes were only non-specific the patient was included in the study only if the clinical picture was characteristic of infarction and attended by appreciable changes in the laboratory tests and if no other disease could be observed to be responsible for these changes

The severity of myocardial infarction was graded by a coronary prognostic index described by Peel *et al* (1962)

Comments The clinical picture of myocardial infarction may as is well known be exceedingly varied The chest pain generally lasts over half an hour but the duration can be shorter or the pain may not be present at all in as many as 20 to 25 % of the patients (Evans and Sutton 1956 Lee *et al* 1957 Johnson *et al* 1959 Stokes and Dawber 1959 Lindberg *et al* 1960) Painless infarction is especially common in elderly patients (Pathy 1967) The pain may be quite severe or take the form only of a vague feeling of pressure or discomfort, the radiation of the pain may be atypical or lacking Painless myocardial infarction may manifest itself as arrhythmia sudden heart failure, collapse or a cerebrovascular accident. Accordingly patients admitted to hospital with these symptoms as well were kept under observation at first for possible definite signs of myocardial infarction

Angina pectoris is defined as a clinical symptom complex produced by an insufficient blood supply to the myocardium but without clearly noticeable myocardial necrosis with its distinctive clinical picture

Typical of the clinical picture are brief paroxysmal substernal pain or pressure attacks provoked by physical or emotional effort and relieved by nitroglycerin

In the present study the foregoing definition was used emphasis was laid on the association with physical or emotional stress although the character of the pain sometimes deviated from the usual.

Coronary insufficiency preinfarction angina, intermittent coronary syndrome, is a poorly defined condition as a clinicopathological concept which has been regarded as a transitional form between myocardial infarction and angina pectoris Physiologically coronary insufficiency is simply a deficient capacity of the coronary blood vessels — for a multitude of reasons — to carry out their function

of transporting blood to the myocardium. It may of course vary in degree and the result range from massive necrosis of the myocardium with the characteristic clinical picture to only transient ischemia and angina pectoris.

In this study the clinical concept of "coronary insufficiency" embraced those patients whose chest pain of more than 20—30 minutes duration lasted longer than in angina pectoris but who lacked the laboratory signs of myocardial infarction and who showed only non-specific ST-segment and/or T wave changes electrocardiographically, which may be due to subendocardial ischemia as well as necrosis.

Comments. Dividing patients into clearly defined angina pectoris and myocardial infarction categories is not always easy although it would, of course, be desirable. It is understandable that in the clinico-pathological picture there must be a sliding scale of changes ranging from an ischemic myocardium to an extensive necrosis. Lack of blood supply over a sufficiently long period of time may cause only diffuse, generally subendocardially situated minor patches of necrosis (Buchner et al. 1935, Snow et al. 1956, Wood 1961, Resnik 1962, Allison et al. 1963, Proger and Naima 1963, Pruitt et al. 1963, Logue and Hurst 1966 b), which fail, however, to appear in the clinical picture as changes definitely distinguishable from ischemia.

Owing to the vagueness of the clinical concept these patients have, however, been omitted from the diagnostic sphere of myocardial infarction in the present study in the knowledge of the evident possibilities of errors in selection.

Heart failure is defined as a deficient capacity of the heart to pump blood in relation to the metabolic needs of the tissues and the amount of venous return. By *congestive heart failure* is meant the condition in which a pathologic congestion of blood occurs in the veins and capillaries as a consequence of heart failure together with an abnormally profuse transmission of fluid into the tissues, mainly owing to increased hydrostatic pressure.

Clinically, in right-sided heart failure, the venous blood accumulating behind the heart stretches the veins and capillaries of the systemic circulation, the venous pressure rises because of this and, in part, of the compensatory sympathetic-isotonic venoconstriction. Increase in the tissue fluid is manifested as edema. In left-sided heart failure the distention of the pulmonary veins and capillaries is followed by transudation of fluid into the interstitium of the pulmonary tissue, stiffening it, or into the alveoles, resulting in pulmonary edema unless the capacity of the lymphatic circulation suffices to remove the excessive fluid.

In the present study bedside signs of congestive heart failure were interpreted as follows. Clinical signs ascribed to congestive heart failure of left ventricular origin: dyspnea at rest, orthopnea and and attacks of nocturnal dyspnea, attacks of wheezing in the absence of pulmonary disease, persistent sinus tachycardia of over 110 beats/min. Signs of pulmonary edema: rapid forced respiration, cough with or without frothy blood and moist rales over entire lung fields. Clinical signs ascribed to congestive heart failure of right ventricular origin: rise of jugular venous pressure over 5 cm above the level of the sternal angle, enlarged, often tender liver and peripheral sacral or ankle edema in the absence of a local disease.

Comments. Transient or persistent left ventricular failure very often occurs in connection with myocardial infarction. This is easily apt to remain unrecognized especially at an acute stage on account of the patient's critical condition. For this reason special care was taken to detect moist rales by careful listening, especially to the lower parts of the lungs, turning the patient slightly on his side. An effort was made not to misinterpret the rhonchi of bronchial secretions often heard during the first respiratory excursions as moist rales. As this investigation included patients with acute myocardial infarction often developing mitral incompetence, the otherwise reliable sign of ventricular gallop (Lewis 1962) could not be used to denote myocardial failure.

When the histories were recorded, the cardiac functional capacity of the patients was graded I to IV by the criteria of the N.Y.H.A. (1953). It is obvious that some overlapping is possible between the symptoms of congestive heart failure and of angina pectoris, as exertional dyspnea can be only a subjective symptom of both conditions (Gilchrist 1951, Muller and Rorvik 1958, Pathy 1967).

Shock is defined as a state of circulatory failure in which the cardiovascular system is incapable of meeting the metabolic needs of the cells. In *cardiogenic shock* the deficiency of the peripheral circulation is due to weakness of the heart as well as to reflex vasoconstriction. The clinical picture is characterized by systemic arterial hypotension, peripheral vasoconstriction and the signs of poor tissue perfusion resulting from them.

In this study the clinical criteria of shock applied were lowering of the systolic blood pressure below 90 mm Hg for over 30 minutes, together with one or more of the following signs: pallor, cyanosis, cold and moist skin, feeble pulse, restlessness and confusion, decreased urinary output. Shock was graded as mild, moderate and severe and scored according to Peel et al. (1962) as 1, 5 or 7.

Comments. Definitions of the concept and degree of severity of shock vary (see discussion in chapter VII). Scored 1, shock in the present investigation was not always accompanied by a decrease of systolic blood pressure below 90 mm Hg, but was transient, disappearing within 15 to 30 minutes. The states of shock scored 5 and 7 were unequivocal in the present writer's view, the score of 7 being

used when vasopressor drugs were given as therapeutic agents

Hypertension is defined as a symptom characterized by abnormally high systemic arterial pressure which is considered to be associated with pathological alterations of the cardiovascular system. Clinically high arterial pressure does not generally bring on symptoms until provoked by complications in the cardiovascular system. No universal agreement regarding the borderline between normal and pathologic values exists (Humerfelt 1963). Blood pressure readings regarded as normal values rise with age (Master and Lasser 1961)

In the present study a patient was considered to have hypertension if values exceeding 160/100 mm Hg were repeatedly measured in hospital or if anamnestic data disclosed hypertension and its magnitude and the mode of treatment

Comments The initial phase of myocardial infarction is often associated with considerable increase of systemic arterial pressure (Wright *et al* 1954 Gilbert *et al* 1954) or more usually a decrease (Laubry and Soulié 1930 Wright *et al* 1954) frequently to the point of a state of shock. The systemic arterial pressure of a patient afflicted with hypertension can become normal after infarction although usually in about two-thirds of the patients a slow rise back to the pathological level takes place gradually (Wright *et al* 1954 Ball *et al* 1955 Friedberg 1966). For this reason blood pressure values recorded only in hospital particularly those measured only occasionally are apt to give a misleading idea of the prevalence of hypertension among infarction patients

Paradoxical cardiac pulsation is a clinical finding which is associated with ischemic partial loss of contractility of the myocardium and with myocardial insufficiency

In palpation the paradoxical pulsation is recognized in the precordium as a systolic outward movement instead of the normal retraction separate from the apex beat. In ischemic damage it is most easily found between the third and fourth ribs midway between the medioclavicular line and the sternum sometimes it may be found near the epigastrium and sometimes only as a sustained heaving apical impulse in which case it is impossible to distinguish from the change of the apical impulse associated with hypertrophy of the left ventricle (Davie *et al* 1952)

The normal apical impulse is located at the fifth intercostal space or medially from it or within 10 cm to the left of the midline. It is felt as a slight brief early systolic thrust (Davie *et al* 1962 Rorvik 1963). With enlargement of the heart it shifts laterally. In hypertrophy of the left ventricle it takes on an even pansystolic heave without any abrupt initial thrust

In the present study a paradoxical pulsation was deemed to be present if it was found to be distinctly separate from the apical impulse or outside its assumed location and to be a pansystolic or almost pansystolic local movement of the precordium causing an outward bulge. A heaving apical impulse was not classified as a paradoxical pulsation except when the systolic bulge accompanying it reached up to the third intercostal space between the medioclavicular line and the midline. Any uncertain palpation finding was noted as negative. The location of the apical impulse was measured from its midpoint. The precordium was examined by lightly pressing with the palm of the hand against it during a held expiratory apnea

Comments The critically important factor in recognizing a paradoxical pulsation is in the present author's view palpation of the precordium by applying a careful and correct technique. It can be detected through painstaking examination in myocardial infarction much more frequently than usually reported (Vakil 1955 Logue and Hurst 1966 b) as also pointed out by Suh and Eddleman (1959). Differentiation between an apical impulse and a left parasternal lift caused by right ventricular hypertrophy is not generally difficult (Logan *et al* 1967)

Mitral incompetence is produced by systolic regurgitation of blood through the leaking left atrioventricular valve from the left ventricle into the left atrium

Systolic murmur of mitral incompetence is the hallmark of mitral incompetence and it may be the only sign of slight incompetence of the mitral valve which otherwise might because of its slightness, be demonstrable only by cardiac catheterization and cineradioangiography of the left ventricle (Leighton *et al* 1966). A murmur is generated during the regurgitant flow vibrations in the wake of the jet being forced back into the atrium (Bruns 1959). The typical murmur of mitral incompetence is high pitched and pansystolic starting immediately from first heart sound and continuing to the aortic component of second heart sound or beyond it because the great pressure gradient and thereby the regurgitation of blood between the left ventricle and the left atrium continues throughout the entire systole including the isovolumetric contraction and relaxation (Leatham 1953 a b Bruns 1959 Perloff and Harvey 1952 Bleifer *et al* 1960)

The murmur is apical local or radiating toward the axilla and softened in inspiration. A pansystolic murmur may vary in shape. Sometimes the murmur starts immediately with first heart sound but fills only the early and mid portion of systole (protomesosystolic) tapering off before closure of the aortic valve (Bridgen and Leatham 1953 Holldack and Wolf 1966 Ilmurzynska 1966). Occasionally the murmur of mitral incompetence may be purely late systolic when associated with papillary-chordal disease (Phillips *et al* 1963 a, Barlow *et al* 1963 Humphries 1964 Segal and Likoff 1964) or of ejection type located more

centrally at the left sternal border (Phillips *et al* 1963 a Luisada 1965 Criley *et al* 1966) Sometimes it is transmitted to the aortic area (Osmundson *et al* 1961 Sleeper *et al* 1962 Burchell 1963 Mercer and Walters 1965) The murmur of marked mitral incompetence is generally associated with third heart sound and with short mid diastolic flow murmur

In the present study the appearance of the murmur described in the foregoing after a silent period of varying duration in connection with myocardial infarction was regarded as a sign of mitral incompetence and was the sole criterion in placing patients in group I defined as acute mitral incompetence developing after myocardial infarction (MIMI)

Comments An apical high frequency pansystolic murmur may be regarded as diagnostic to mitral incompetence, although of low intensity (Bridgen and Leatham 1953 Mounsey and Bridgen 1954 Leatham 1958 a b McKusick 1958 Perloff and Harvey 1962 Ravin 1967) Difficulties may arise when the murmur of mitral incompetence rarely presents itself as ejection type and radiates to the aortic area The murmur of *aortic stenosis or sclerosis* (Bruns and van der Hauwaert 1958 Bruns 1959) may again be found also at the apex or only audible there (Wood 1938) This murmur is however generally harsher definitely of the ejection type and usually radiating to the neck In valvular aortic stenosis the ejection click or valvular calcification may help in determining the aortic origin of the murmur The murmur of aortic stenosis is chiefly apical only in the subaortic type of stenosis (Soulie *et al* 1963 Braunwald *et al* 1964) Other positive clinical signs of this disease are usually evident although sometimes differentiation from mitral incompetence is difficult (Raftery *et al* 1966) Moreover mitral incompetence is a usual sequela of hypertrophic muscular subaortic stenosis

Innocent ejection type murmur is usually an early systolic soft ejection type murmur at the left sternal border radiating to the pulmonary area The murmur usually has a rather vague maximal point of intensity It is profoundly affected by respiratory excursions and by changes of body position It does not radiate to the axilla An innocent murmur is rare after childhood and adolescence in older patients generally an extracardial factor causing a hyperkinetic circulatory state or large stroke volume is found such as for instance anaemia tachycardia fever thyreotoxicosis or bradycardia (Leatham 1958 a b Humphries and McKusick 1962 Shabetai 1963 Weaver and Walker 1964)

The murmur of *tricuspid incompetence* is pansystolic but its location is at the lower sternum with increasing intensity in inspiration (Zeh 1959) Positive jugular venous and hepatic pulses are also helpful in diagnosis

Pericardial friction rub is characteristically manifested as rubbing sounds of variable intensity being generally present in both systole and diastole. It is often (10—20 %) found transiently in acute myocardial infarction during the first few days (Hochrein 1937 Mintz and Katz 1947 Yater *et al* 1948 Laubry and Soulié 1950 Wright *et al* 1954 Plotz 1957 Friedberg 1966) Sometimes only a faint high frequency systolic sound of friction is present which is hard to differentiate from a murmur But generally marked alterations in intensity location and timing occur during changes of position or the respiratory phase to become evident in the course of a few days enabling the auscultatory finding to be classified as a pericardial friction rub

It is unavoidable that some overlapping is possible in classifying a faint ejection type medium frequency parasternal or apical murmur as being of aortic or of mitral valve origin especially when the aortic valvular murmur may be inaudible during the sometimes low output state in the initial phase of myocardial infarction (Schimert *et al* 1960 Spencer and Greiss 1962) This applies however only to murmurs of the ejection or short decrescendo type of low intensity Pericardial friction and the innocent ejection murmur of tachycardia as well as the murmur of tricuspid incompetence can usually be differentiated without any difficulty from the murmur of mitral incompetence

STATISTICAL TECHNIQUES

Standard statistical techniques have been used in this study In testing differences between two sample means normal probability function has been applied for large samples *t* ratio for small samples Differences between more than two sample means have been tested with the F test Existence of correlation in contingency tables has been tested by chi square and Fisher exact probability tests The degree of correlation between dichotomous and continuous variables has been measured with a biserial correlation coefficient The sign test based on the binomial distribution has been used in testing significances of class frequencies in one-sample cases Probabilities above 0 05 have been considered not significant.

B CLINICAL EXAMINATION PROCEDURE

All the patients included in the material of the present study were examined by the author personally On the day of admission or the following day a detailed history of earlier cardiovascular diseases was taken and the duration and grade of these disturbances evaluated During hospitalization a clinical examination was performed daily during the first ten days and thereafter every other day until the patient was dis

charged. The average duration of hospitalization was four weeks

A thorough bedside examination was performed to reveal any signs of alterations in the cardiovascular system in the course of treatment espe cially to detect signs of heart murmurs congestive heart failure and paradoxical cardiac pulsation A meticulous auscultation of the entire precordium was performed with the patient in supine and left lateral positions to detect murmurs and gallop sounds as well as changes in first and econd heart sounds. A search for murmurs in the carotid and femoral arteries was also made The presence of arrhythmias was noted When murmurs were found they were recorded by direct writing external phonocardiography if possible

Conventional 12 lead electrocardiograms were taken and laboratory tests made according in the scheme in which an electrocardiogram was taken and the leucocytes and serum GOT and LDH were determined every day during the first four days in hospital and thereafter every fifth day until the patient was discharged. The ESR was determined on the first third and fifth days in hospital and thereafter together with other tests

Routine radiological examinations of the heart and lungs were performed on all patients in the Department of Radiology by means of postero-anterior and lateral pictures 2—4 weeks after admission

MATERIAL

A PATIENTS

Clinical study of the patients during the acute phase of myocardial infarction was performed between June 1965 and March 1966 The patients had been admitted to the First and Second Medical Clinics of the University of Helsinki, and to two male wards of the Third Medical Clinic of the University of Helsinki

The majority of the patients arrived at the hospital from the city or its environs within a few hours after the appearance of the subjective symptoms

In order to obtain the most reliable possible picture of the incidence of mitral incompetence brought on by acute myocardial infarction it is obviously important to avoid selection of the patient series With this in mind, the author at first included unselectively for the purposes of investigation and examined all the patients consecutively admitted to hospital as actual or suspected cases of myocardial infarction

If the possibility of the myocardial infarction was considered after the initial examination but the clinical picture remained doubtful the patient was tentatively included in the examination procedure for about a week to verify the presence or absence of acute myocardial infarction by means of the clinical course, the pattern of serial electrocardiography and by means of the data from the clinical laboratory If the criteria of myocardial infarction as defined in the foregoing (Chapter IV were not met the patient was excluded from further observation and inclusion in the patient series

For every patient, the author himself decided whether the results of the examinations met the criteria of infarction, regardless of the stand taken by the physician in attendance

Comments Owing to the character of ischemic heart disease all the clinical materials relating to myocardial infarction are more or less selective The first sign of acute coronary disease may be sudden death caused by cardiac arrest, so naturally such patients never reach the hospital for examination A small proportion of the patients do not apply for admission because of the slightness of their symptoms In some 15 % of the patients, myocardial infarction develops without typical symptoms A painless, "mute infarction is apt to be wholly overlooked or to manifest itself as arrhythmia, acute heart failure, collapse or an acute cerebrovascular accident (Pathy 1967) In order to avoid the possibility of this unintentional selection, the patients presenting these symptoms were also kept under observation for a week to discover a possible myocardial infarction

It is often difficult to draw the line in diagnosis between myocardial infarction proper and prolonged angina pectoris or ,acute coronary insufficiency and selection in this respect could substantially alter the character of the myocardial infarction material being investigated The criteria used to solve this problem in the present investigation are presented in the preceding chapter

EXCLUDED PATIENTS

Patients were not included in the investigation if

— more than three days had passed since the estimated onset of the infarction before admission to hospital

— the patient died of acute myocardial infarction before three days of hospital observation had passed

— the afore mentioned criteria applied to acute myocardial infarction were not met

B GROUPING BY AUSCULTATORY FINDINGS

The total number of patients admitted to the various wards for treatment of actual or suspected myocardial infarction and initially kept under observation for a week or less was 306 Of these initially observed subjects, 96 were excluded from the patient series analyzed in this study because they did not meet the requirements of infarction diagnosis or for other reasons described in the preceding section

The series clinically studied thus comprises 210 patients with acute myocardial infarction The patients were placed in the following five groups according to their earlier history and on the basis of auscultatory observations made during the course of examinations

Group 1 consisted of 117 patients with acute mitral incompetence as a complication of acute myocardial infarction the MIMI group The constant or transient murmur of mitral incompetence described in the previous chapter, served as a criterion

Group 2 consisted of 70 patients who had no heart murmur excluding pericardial friction or had developed none during their stay in hospital

Group 3 consisted of 6 patients who had a constant murmur of mitral incompetence on admission which could be thought to have originated in conjunction with an earlier infarction

Group 4 comprised 7 patients who since admission had also had a constant murmur of mitral incompetence, the etiology of which could not be traced on the basis of the available information

Group 5 comprised 10 patients who had loud precordial murmur of aortic stenosis or aortic sclerosis so that it was not possible clinically to find the possible development or existence of faint mitral systolic murmur

The distribution of the patient material into the five groups mentioned is shown

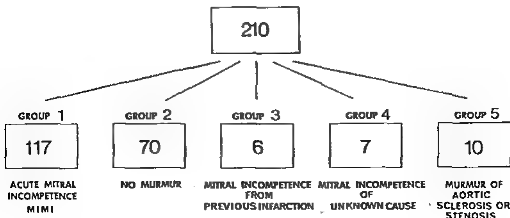


Fig 1 *Presentation of the patient series*

in Fig 1 It is self evident that a closer study of groups 3—5 would not have shed additional light on the characteristics of acute mitral incompetence occurring as a complication of myocardial infarction Accordingly, the later detailed analysis was carried out with groups 1 and 2 which together included 187 patients

CONTROL PATIENTS

As stated, the object of this study was to investigate a certain complication of myocardial infarction, the prevalence and characteristics of the mitral incompetence occurring as a result of myocardial infarction Therefore, there exists no control patient material parallel with the myocardial infarction material The "control patients" thus perforce consisted of patients characterized by the presence of acute myocardial infarction without the systolic murmur of mitral incompetence on admission or during the course of hospital treatment

C AGE AND SEX DISTRIBUTION

Age distribution in the patient groups is shown in Fig 2 and in Tables 2 and 3 The distribution of patients with and without mitral incompetence according to age was quite similar in both groups the cumulative age distribution curves being almost identical

The number of males and females appears in Table 3, which shows their distribution into different age groups In males the incidence of acute MIMI was highly significantly ($p < 0.001$) lower than in females

Table 2 *Age distribution in the patient groups*

Group	No of patients	Age years Range	Mean
MIMI	117	33—78	55 3
No murmur	70	36—85	57 3
Total series	210	33—86	56 9

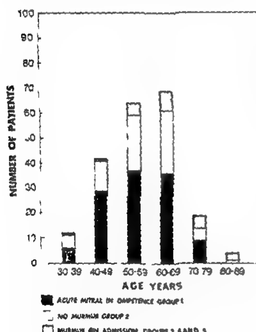


Fig 2 *Age distribution in the patient groups*

Table 3 *Distribution according to age and sex in patients with and without mitral incompetence*

Sex	Age years 30 39	40 -49	50—59	60 -69	70 -79	80—89	Total	
Males								
Mitral incompetence	5	24	30	25	7	[illegible]	91	(58 %)
No murmur	5	12	21	22	4	1	65	(42 %)
Females								
Mitral incompetence	1	5	6	12	2	0	26	(84 %)
No murmur	0	0	1	3	1	0	5	(16 %)

AUSCULTATORY AND PHONOCARDIOGRAPHIC CHARACTERISTICS OF MITRAL SYSTOLIC MURMUR

METHODS

A AUSCULTATION

GENERATION OF MURMURS

Murmurs result from pressure gradients which are capable of accelerating the flow of blood to the extent of creating vortices Such vortices and almost periodic fluctuations of blood downstream from any obstacle as well as perhaps flutter mechanism are considered to be responsible for most murmurs (McDonald 1952 Bruns 1961 Laurens 1964 Rodbard 1964) although some aspects continue to be somewhat controversial

Factors intimately related to murmur production include the diameter of the flow channel and sudden even though slight changes of this diameter the viscosity of the blood and one of the most important determinants is the stream velocity The stream velocity in turn is directly dependent on the square root of the pressure gradient which again depends on the volume flow per unit cross section area of the orifice (Rodbard 1964)

CLASSIFICATION OF MITRAL SYSTOLIC MURMURS

Leatham (1958 a b) has stressed the close dependence of the pattern of the systolic murmur on the quality and quantity of hemodynamic alterations Clinically the classification of the type of systolic murmur is based on the timing of the murmur in relation to first and second heart sounds and on its intensity in various parts of systole

Murmurs are broadly classified in pansystolic regurgitant and in ejection type murmurs When pansystolic murmur occurs regurgitation of blood takes place during systole from a high pressure area back to a low pressure area The pressure gradient already exists during the isovolumetric contraction and extends to the isovolumetric relaxation thus causing a murmur throughout the whole systole

Ejection type systolic murmurs do not otherwise fill the whole systole They indicate an obstruction in the flow of blood or an acceleration of the velocity of the blood stream during the early systolic ejection Pansystolic murmurs are thus invariably pathological the ejection type may also be innocent as a result of an increase in cardiac output and/or stroke volume for physiological reasons

TYPE OF MURMUR

Mitral systolic murmurs are classified in different ways (Luisada 1965 Holldack and Wolf 1966) depending on the timing of the murmur and the position of the peak intensity in systole Perloff and Harvey (1962) classified mitral systolic murmurs into six types according to phonocardiographic findings

In the present study mitral systolic murmurs are classified into three main types and eight subgroups on the basis of the timing and shape of the murmur

Timing The *pansystolic* murmur fills the whole systole, starting immediately from first heart sound and continuing to second sound usually an aortic component at the apex or beyond.

A murmur is defined as *protomesosystolic* (early mid-systolic) if the beginning of the murmur is not separated from first heart sound but ceases definitely before second heart sound On the whole this type of protomesosystolic murmur may also be included in the pansystolic group on account of its generative mechanism since the murmur begins as early as the stage of isovolumetric contraction

The *ejection type* murmur begins after a silent delay following first heart sound and is peaked or diamond shaped.

Form Pansystolic murmurs were further classified according to their form into plateau (even intensity) crescendo (increasing) decrescendo (decreasing) and crescendo-decrescendo (peaking) types

The protomesosystolic murmurs were classified into decrescendo and crescendo-decrescendo types according to their form.

The subgrouping of the ejection type murmur was not based on form but on timing which was either early systolic (ending before second heart sound) or late systolic (continuing to second heart sound)

The classification of murmurs according to the timing and form of the murmurs into main

and sub-groups ■ shown in Fig 3
The pattern of murmurs found in auscultation was used in recording the results

PANSYSTOLIC

BLATEAU



CRESCENDO



DECRESCENDO



CRESCENDO-DECRESCENDO



PROTOMESOSYSTOLIC

DECRESCENDO



CRESCENDO-DECRESCENDO



EJECTION

EARLY



LATE



Fig 3 *Classification of mi ral systolic murmurs*

OTHER MURMUR CHARACTERISTICS

The *location* and *transmission direction* of a murmur are quite helpful in clinical evaluation of the origin of the murmur In determining the location and transmission generally accepted principles were applied (Shah *et al* 1964)

The determination of the *intensity* of the murmur is based on arbitrary classification and ■ includes possibilities of error associated with the characteristics of hearing Matters are no surer however in phonocardiography In the present study the scale of 1 to 6 for murmur intensity as presented by Levine and Harvey (1959) was adopted. It has generally been found to be fairly reliable after gaining experience of it

The *frequency* of the murmur was defined as low medium or high

FACTORS LIMITING ACCURACY OF AUSCULTATION

Clinical auscultation is a subjective auditory sensation and it involves the interpretation of the sounds and murmurs detected in the subjects examined Thus many of the characteristics of the subject the stethoscope and the observer affect the accuracy of the au cultation finding

Sound transmission The transmission of a sound from its site of origin in the heart or blo d vessel to the chest depends on many to some extent poorly known factors These vary from person to person (see McKusick 1958 Ongley *et al* 1960 Luisada 1965) The sound travels in the blood in the direction of moving at the time of its production in the cardiovascular system The sound also radiates into the surrounding tissues Before its arrival at the skin of the chest the heart sound or murmur is damped by the different transmission characteristics of tissues and is reflected in the interfaces of the thoracic structures The intensity decreases in direct proportion to the square of distance The structure of the chest and the thickness of the subcutaneous fat the position of the heart in the chest diseases of the cardiopulmonary system such as fluid effusions and emphysema thus appreciably affect the transmission of sounds Not only the intensity of the murmur but also its frequency and duration are apt to change during the transmission Accordingly the intra and extracardial phonocardiographic findings may vary in the same subject and vary even more from one patient to another Transmission of high frequency murmurs is poor and these may be only weakly de ected intracardially (Segal *et al* 1964 Leighton *et al* 1966) Best transmitted are the 150 – 350 cps sounds (Feruglio 1962 Zalter *et al* 1963) Deep inspiration attenuated the murmur intensity by 50 % and in expiration an increase of 15 % over apnea or quiet respiration was found in a study where an intracardiac sound generator was used in man (Feruglio 1962) The effect of changing distance was probably an important factor

The quality of the stethoscope is an important factor in auscultation A Rappaport Sprague stethoscope equipped with an open bell and with a diaphragm end piece was used in the present study (Rappaport and Sprague 1941 Ongley *et al* 1960)

Auditory system It ■ often forgotten that the basic instrument of auscultation is the human ear (Luisada 1965) The total vibration energy generated by the action of the heart occurs mainly in the low frequency area (Butterworth and Keppert 1966) and only about 10 % lies above the auditory threshold An important part of the vibrations is thus subliminal and inaudible which otherwise can be felt by palpation or registered as ultra low frequency displacement or acceleration tracing Although the human auditory range is 16 – 16 000 cps maximum hearing sensitivity is limited to

the area between 1 000 and 2 000 cps (Fletcher and Munson 1933) Below this accuracy falls rapidly as the range of the heart sounds are reached (10–800) Thus the ear needs 100 times greater sound pressure to hear a sound of 100 cps than one of 1 000 cps Sound pressures of audible heart murmurs are about 25–65 decibels the maximum sensitivity of the ear in this area is at 100 cps (Rappaport and Sprague 1942 Butterworth *et al* 1960 Ongley *et al* 1960)

In the hearing ability of the ear there is a logarithmic distortion so that with sounds of the same intensity those of higher frequency seem louder than hose of lower frequency Thus comparing the intensities of high and low frequency heart murmurs involves certain difficulties

On the other hand the human hearing mechanism is capable of selective interpretation as are other sensory systems Otherwise the tremendous constant sensory input would quickly tire the integrating centres of the brain (Livingston 1959) This ability to perceive selectively helps greatly in blocking out disturbing and meaningless perception such as that of respiratory sighs and background noise when listening to weak heart sounds and murmurs

The varying characteristics of the auditory system in different observers may explain some of the differences in auscultation findings

Background noise The level of background noise can profoundly alter the recognition of heart sounds and murmurs especially if the observer is not particularly concentrating on listening According to the studies of Groom (1956) background noise is constantly 60–70 decibels in clinical and hospital wards Groom points out that a sound heard in a quiet room would have to be amplified 12 times in order to become audible under the average noise conditions in a hospital ward Obviously this phenomenon was a factor of the utmost importance in the present investigation when heart sounds and murmurs often of poor magnitude were studied

TECHNIQUE OF AUSCULTATION

The art of auscultation is not an inherent skill Careful technique an ability to concentrate and to tune in to selective auscultation procedure and the interpretation of findings in terms of altered pathophysiology are acquired only by thorough training as has repeatedly been emphasized (Levine and Harvey 1959 Ongley *et al* 1960 Harvey 1964 Ravin 1967) These factors are obviously important in obtaining the full value of auscultation as a method of bedside examination

AUSCULTATION PROCEDURE

The patients were examined daily during their first ten days in hospital and thereafter every second day until discharged First the precordium was palpated for the apical impulse and any paradoxical pulsation or palpable shock of third heart sound After the first examination of the patient in the supine position he was turned to the left lateral position and the apical area was again thoroughly auscultated It was sometimes only in this way that a faint systolic apical murmur or gallop sound could be recognized Simple measures to diminish ward noise were taken whenever possible The selective auscultation method was used concentrating only on one part of the heart cycle at a time The carotid arteries were auscultated Then the character of first heart sound was studied and compared to second sound at the apex The intensity of the aortic and pulmonary components of second heart sound was noted with the diaphragm at the left sternal border in the third intercostal space The splitting of second heart sound was carefully sought during slow deep respiratory excursions Broader splitting than the usual physiological respiratory splitting was considered pathological Then the lower precordium was examined with the bell of a stethoscope to detect any third or fourth heart sound The bell was applied to the skin with only very light pressure to prevent any air leak which is often a critical measure in detecting weak gallop sounds The left lateral parasternal area was auscultated with the diaphragm with firm pressure to discover regurgitant murmurs of the semilunar valves Finally a meticulous search was made for systolic murmurs in the parasternal and apical areas Sometimes an apical thrust caused a systolic thudding sound when the bell end piece was used but with the diaphragm this auscultatory artifact was easily excluded The type of murmur was plotted as were also any changes occurring in the murmur pattern during the course of observation

B PHONOCARDIOGRAPHY

APPARATUS

All the phonocardiograms were recorded with the same type of apparatus Mingograph 31 B (Elema Schonander Ab Sweden) The filters are of the high pass type and the apparatus has an amplifying system to compensate for the lowering of intensity when the frequency of the murmur rises The direct jet ink writer apparatus has a linear response up to 500 cps The nominal frequencies of the filters were 35 70 140 (logarithmic) and 250 cps Phonocardiograms were recorded by all the filters every time to obtain selective sensitivity for both low frequency (third and fourth heart sounds) and high frequency (systolic murmurs) phenomena on recording (Fig 4) The paper speed was 100 mm sec. or sometimes 50 mm/sec.

Phonocardiograms of the pharmacodynamic investigation carried out in connection with the cardiac catheterization were recorded by Sanborn devices described in detail in Chapter VIII

RECORDING PROCEDURE

All the phonocardiograms were recorded by the author This was done whenever possible when a murmur was detected. For the purpose of ascertaining the intra-observer error in auscultation the murmur was drawn on paper and the presence or absence of third and fourth heart sound was noted before recording As recordings were usually made only to document an apical murmur of mitral incompetence developing after myocardial infarction this phonocardiographic comparison of increased intensity or splitting of second heart sound is unfortunately not available An air transmission type microphone was lightly held by hand at the point of maximal intensity of murmur Often the patient had to be turned to the left lateral position to obtain a better murmur amplitude in the recording Appropriate amplification was chosen to gain a clear picture of the murmur type Usually maximum amplification that did not however distort the base line by an inappropriate signal noise ratio had to be used because of a faint high frequency systolic murmur The patient was asked to stop respiration after normal expiration for a while during recording A standard lead II of an electrocardiogram was always used as a reference recording

INTERPRETATION

In the interpretation of phonocardiographic recordings the murmur pattern was determined from several heart cycles Care was taken not to interpret artifacts such as respiratory sighs as a murmur This was easily accomplished by examining several cardiac cycles in which constant time relations and form of murmur were clearly visible The same method was employed in recognizing third and fourth sounds Rather faint fourth sounds were also noted The same classification as in auscultation was used for the murmur types

ACCURACY OF PHONOCARDIOGRAPHY

The same limiting factors of sound transmission as those discussed under the heading of auscultation also apply to phonocardiography Thus murmurs registered by intracardiac phonocardiography may not to be recorded by external phonocardiography (Segal *et al* 1964 Leighton *et al* 1966) and the murmur pattern may be distorted during transmission to the wall of the chest (Leighton *et al* 1966) The sharply filtered band of the phonocardiograph may take only a small spectrum of murmur vibrations and so change the type as compared to the auscultatory finding (Fig 5) In this study it also became clearly evident that the ear is more sensitive than phonocardiography in the recognition of murmurs of poor magnitude when of high frequency This is also mentioned by Schrire *et al* (1961) Friedberg (1966) and Luisada and DiBartolo (1961) who used special experimental phonocardiograph apparatus to solve this registration problem Many times great difficulties were encountered in recording a pansystolic faint but distinctly and unmistakably audible high frequency murmur On the other hand owing to the characteristics of phonocardiographs and the human auditory system there is no problem in obtaining recordings of low frequency third or fourth heart sounds barely audible in auscultation

From the point of view of method it is obvious that although phonocardiographic records were taken only for documentation if a murmur was found to have developed no external phono

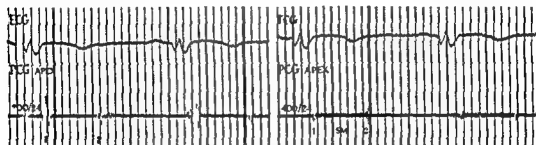


Fig 4 *Phonocardiograms showing the importance of filter selection for recording a faint murmur of high frequency With a 200 cps high pass filter band the murmur grade 2 in auscultation was not registered at all but is clearly present in the recording made with a 400 cps filter band*

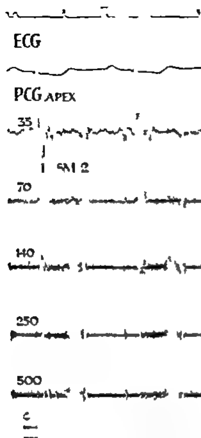


Fig 5 *Phonocardiogram showing a marked ex clusively h gh frequency late systolic crescendo of a pansystolic murmur of mitral incompetence*

cardiography would have detected any high-frequency mitral systolic murmurs more sens tively than careful auscultation Equally obviously matters are reversed when third and fourth heart sounds are stud ed. In 211 comparisons 8 % of third and 10 % of fourth heart sounds present in phonocardiography were missed by auscultation. The type of murmur was misinterpreted in 6 instances

A large proport on of the murmurs of poor magnitude made class fication of the type of mitral systol c murmur by the method of Perloff and Harvey (1962) impracticable in this study and therefore the same class fication as in auscultation was used n the phonocardiographic interpretations.

RESULTS

1 INCIDENCE OF ACUTE MITRAL SYSTOLIC MURMUR (Fig 1)

A permanent or transient murmur con sistent with mitral incompetence resulting from acute myocardial infarction (MIMI) developed in 117 patients during hospital observation In 70 patients no murmurs were detected throughout this time with the exception of a few constant carotid systolic murmurs which could not be heard outside the base of the heart, and one patient (No 164) with a murmur of mild aortic insufficiency One patient (No 180) who developed tricuspid in competence (section 7) was included in the no-murmur group Patient groups 3, 4 and 5 (Fig 1) contained in the total material are not analyzable when esti mating the incidence of acute mitral incompetence as a complication of myocardial infarction. Accordingly, the per centage of mitral incompetence is 63 % of the 187 patients in groups 1 and 2, and 56 % of the total material The true inci dence of MIMI in the present material thus falls somewhere between these two percentage figures

Pericardial friction rub was observed in 14 patients of the MIMI group and in 5 no-murmur patients

2. TIME OF ONSET OF MITRAL SYSTOLIC MURMURS (Fig. 6)

All mitral systolic murmurs The first day of its being noticed was taken as the time of onset of a murmur from the estimated onset of myocardial infarction. The time of onset of the murmurs for the entire mitral incompetence group was mainly within the first 5 days of illness After that the murmur appeared fairly often between the 6th and 9th days but thereafter only seldom During the first 10 days 87 % of the murmurs had appeared and thus during the remaining time only 13 % The first day included 7 patients who already had a murmur on

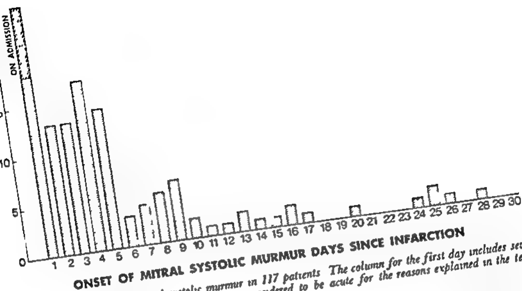


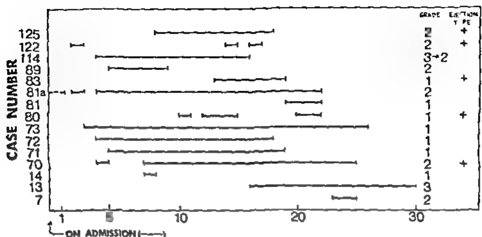
Fig 6 *Time of onset of mitral systolic murmur in 117 patients The column for the first day includes seven patients with murmur present on admission but considered to be acute for the reasons explained in the text*

admission but 4 of these were classified as acute MIMI (Case Nos 3, 32, 87 and 108) on the basis of subsequent disappearance and re appearance, (Fig 7) and 3 (Nos 8, 10 15) because the autopsy examination revealed no other alteration except severe fresh destruction of pillary muscle as an explanation of mitral systolic murmur

Transient and inconstant mitral systolic murmurs (Fig 7) The time of onset of transient murmurs did not differ from that of constant murmurs The duration of a transient murmur varied from 1 to 24 days, the mean being 10 days The group with an inconstant murmur consists of the patients in whom the murmur could not be heard continuously during the observation period but in whom there were silent periods of varying duration However, the murmur was still present on the patient s discharge from hospital Fig 7 indicates the variation in time of onset and the duration of the silent periods The principal trend was a murmur of brief duration with an early appearance, which after a few days re appeared as constant and often as a different type (see section 4)

3 MAIN CHARACTERISTICS OF MURMURS (Table 4)

Prevalent type The prevalent type of murmur in the MIMI group was chiefly pansystolic (91 %), and only a small proportion of the murmurs were mainly protomesosystolic (3 patients) or of the ejection type (7 patients), Fig 3 Otherwise ejection type murmur occurred often as a variant type (section 4) Of the sub types, the pansystolic plateau type (Fig 8) was overwhelmingly the most prevalent occurring more often than all the other types combined (57 %) Next most common among the sub types was the pan systolic decrescendo (Fig 9), which accounted for 20 %, followed by the crescendo type (Fig 10), with 9 % No mainly decrescendo type of protomeso-systolic murmur was observed, all 3



CASE NUMBER
123 120 117 116 108 106 105 97 94 93 87 82 78 75 69 63 61 60 58 57 55 51 34 32 17 9 3
GRADE
EJECTION → PANSYSTOLIC
1 5 10 20 30
ON ADMISSION (----)

MURMUR PRESENT DAYS SINCE INFARCTION

Fig 7 *Occurrence of the transient (upper group) and inconstant (lower group) mitral systolic murmurs The presence of only an ejection type murmur in the group of transient murmurs (in 2 patients varying d crescendo or ejection type) or the change of an initially ejection type murmur to a pansystolic one in the group of inconstant murmurs is indicated by +*

Table 4 *Main characteristics of mitral systolic murmur at hospital*

Type	Intensity 1	2	3	4	Location Apex	LSB	Apex LSB	Transmission Axilla	LSB	None	Frequency High	Medium	Permanence Constant	Inconstant	Transient	Total
Pansystolic																
Plateau	8	19	18	4	65	1	1	15*	20*	37	51**	13	15	16	6	67 (57 %)
Crescendo	1	3	5	2	11	0	0	7	3	1	11	0	8	3	0	11 (9 %)
Decrescendo	9	13	1	0	19	1	3	2	6	15	17	6	12	6	5	23 (20 %)
Cresc decresc	0	5	1	0	6	0	0	3*	3*	2	4	2	1	1	1	6 (5 %)
Total	18 (15 %)	60 (51 %)	25 (21 %)	4 (3 %)	101 (86 %)	2 (2 %)	4 (3 %)	27* (23 %)	32* (27 %)	[illegible] (47 %)	86** (74 %)	21 (18 %)	[illegible] (59 %)	26 (22 %)	12 (10 %)	107 (91 %)
Protomesosystolic																
Decrescendo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0 %)
Cresc decresc	0	0	3	0	1	1	1	0	1	2	1	2	3	0	0	3 (3 %)
Total	0	0	3	0	1	1	1	0	1	2	1	2	3	0	0	3 (3 %)
Ejection																
Early	2	2	2	0	4	1	1	0	4	2	3	3	3	1	2	6 (5 %)
Late	0	1	0	0	0	0	1	0	0	1	0	1	0	0	1	1 (1 %)
Total	2	[illegible]	2	0	4	1	2	0	4	3	3	4	3	1	[illegible]	7 (6 %)
Total	[illegible] (17 %)	63 (54 %)	30 (26 %)	4 (3 %)	106 (91 %)	4 (3 %)	7 (6 %)	27* (23 %)	37* (32 %)	60 (51 %)	90** (77 %)	[illegible] (23 %)	75 (64 %)	27*** (23 %)	15 (13 %)	117 (100 %)

LSB left sternal border

* Transmission to both axilla and left sternal border in some of patients
** 7 patients with medium high frequency murmur included
*** 5 patients with inconstantly audible transient murmur not included

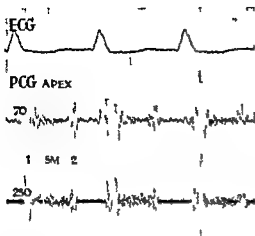
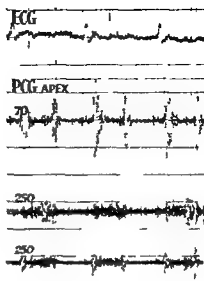


Fig 8 *Phonocardiogram showing the common plateau type high frequency pansystolic murmur of mitral incompetence*



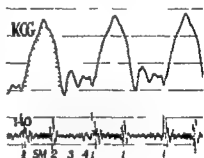


Fig 10 *Phonocardiogram representing the crescendo-type pansystolic murmur This is evident in a tracing obtained through a filter sensitive to high frequencies (250) With a filter of a nominal frequency of 70 cps not sensitive to high frequencies the crescendo character is not recorded A kinetocardiogram (KCG) confirmed the finding of a marked paradoxical cardiac pulsation (stippled area)*

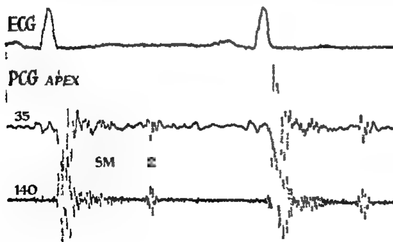


Fig 9 *Phonocardiogram of a decrescendo type mitral systolic murmur pansystolic in auscultation*

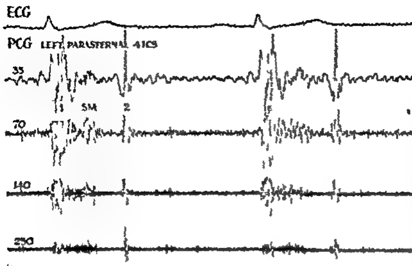


Fig 11 *Phonocardiogram of a protomesosystolic crescendo decrescendo type murmur The murmur changed transiently to a louder pansystolic plateau type several times during several attacks of anginal pain (Table 7 case No 37)*

patients with a protomesosystolic murmur having the crescendo-decrescendo type Fig 11)

It is obvious that the line dividing the decrescendo type of murmur according to pansystolic or protomesosystolic duration must be a somewhat elusive one Phonocardiography did not help deal with this problem since for technical reasons see *Methods)* a faint decrescendo murmur might appear to be protomesosystolic in phonocardiography notwithstanding its extension over the whole systole in auscultation

Ejection type murmurs mainly had a short early systolic timing (Figs 3, 12) A late systolic murmur as the prevalent type was noted in only one patient with a transient murmur of 18 days duration (Fig 13) In one patient the first manifestation of an inconstant murmur was a late systolic murmur, which after lasting a few hours disappeared completely, only to be replaced later the same day by a permanent early systolic or sometimes pansystolic murmur

Prevalent intensity of murmurs (Table 4, Fig 15) In the main the murmurs were of a somewhat low intensity, chiefly grade 2 (54 %) the second most common

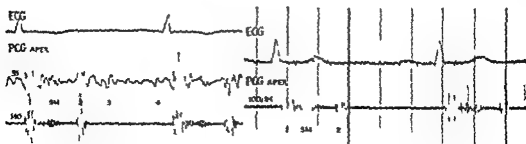


Fig 12 *Two examples of distinct ejection type medium frequency apical mitral systolic murmurs ascribed to an ischemic dysfunction of the papillary muscle Both murmurs later changed to a definitely pansystolic type*

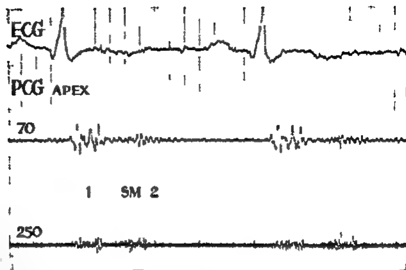


Fig 13 *Phonocardiogram of a purely late systolic type of murmur The murmur disappeared after 18 days*

being grade 3 (26 %), then grade 1 (17 %) and finally grade 4 (3 %) A fairly loud prevalent intensity, grade 3 or over thus occurred in less than a third of the patients (29 %) The final prevalent intensity of murmur varied somewhat so that in grade 1 it was 1—2 (8 patients), in grade 2 2—3 (13 patients), while in grade 3 the murmur occurred occasionally in 5 patients often at grade 4 intensity No murmur over grade 4 in intensity was noted

Pansystolic murmurs were correlated with loud (grade 3 or 4) intensity ($p < 0.01$) whereas this was not the case with the ejection type or protomesosystolic murmur The loud murmurs all those of grade 4 and most of grade 3 were of the pansystolic plateau crescendo or crescendo—decrescendo type (Fig 14) On

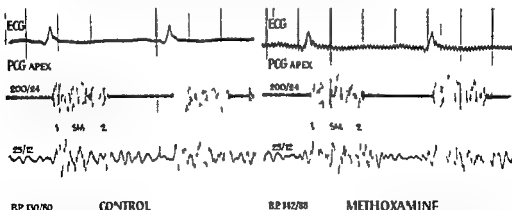


Fig 14 *Phonocardiogram of a crescendo-decrescendo type pansystolic murmur of mitral incompetence The mid systolic peak became more prominent when the intensity of the murmur was increased by an infusion of methoxamine*

the other hand, the intensity of the decrescendo murmurs was only once over grade 2

No correlation was found between murmur intensity and severity of mitral incompetence as judged clinically or by hemodynamic investigation

Relation of the heart volume to the intensity of the murmur showed that in enlarged hearts the murmur was more often loud, 63 % of the grade 3 or 4 murmurs occurred in enlarged hearts while 66 % of the grade 1 or 2 murmurs occurred in normal sized hearts

Location, transmission and frequency of murmurs and their sub types (Table 4) The *location* of mitral systolic murmurs developing as a consequence of acute myocardial infarction was nearly always apical (91 %) This was also true in many instances of the ejection-type murmur Only occasionally was the location at the left sternal border in the third or fourth intercostal space, there were 4 such patients In 7 patients the maximum intensity occurred between the apex and the midline

Transmission of murmur The most common finding was that the murmur was not transmitted (51 %), as may be expected with murmurs of mainly low intensity Transmission was more common to the left sternal border (32 %) than to the axilla (23 %) No protomesosystolic or ejection type murmur was transmitted to the axilla The transmission of a murmur to the left sternal border was sometimes extended to the aortic or pulmonary area The crescendo-type murmur was rather often transmitted to the axilla, and it was invariably apical

High frequency was almost as common a finding in the investigation of the murmurs (77 %) as were pansystolic duration

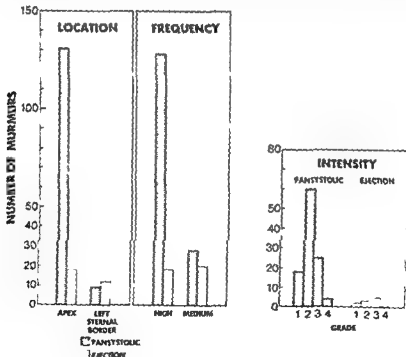


Fig 15 Relation of the murmur type to its location pitch and loudness Ejection-type group includes protomesosystolic murmurs

Table 5 *Relation of intensity of murmur to permanence of murmur*

Permanence	Intensity		Total
	Grade 1 or 2	Grade 3 or 4	
Constant	47 (57 %)	28 (8[illegible] %)	[illegible] (64 %)
Inconstant	22 (26 %)	5 (15 %)	27 (23 %)
Transient	14 (17 %)	1 (3 %)	15 (13 %)
Total	83 (100 %) (71 %)	34 (100 %) (29 %)	117 (100 %)

and apical localization A medium frequency was relatively more common in the ejection type murmurs The crescendo type always had a high frequency

Fig 15 presents a correlation of murmur type with its location and frequency An analysis was made not only of the prevalent characteristics but also of the different types, locations and frequencies of murmurs in the same subject at different times This was done when it became evident during the course of the investigation that the pansystolic murmur was generally apical and of high frequency whereas the ejection type (or protomesosystolic) murmur appeared to be associated more with a parasternal location and to be of medium frequency the type of murmur was even apt to vary in the same patient (see *Appendix*) These definite correlations are apparent from Fig 15 and are highly significant ($p < 0.001$) for the pansystolic murmur In the ejection type or protomesosystolic murmur the distribution was more often in a left sternal border location and of medium frequency, although the difference was not significant

Permanence of murmur (Table 4) The murmur was permanently audible without any disappearence after its development during hospitalization in not more than two-thirds of the patients (64 %) Transient murmurs which disappeared after varying periods (Fig 7) were observed in 15 patients (13 %) The remaining patients had inconstant murmurs and the incidence was 23 % (not including 5 patients with an inconstantly present transient murmur)

Crescendo-type murmurs were never transient Transient murmurs were usually of the plateau, decrescendo or ejection type Constant murmurs were not often of the decrescendo type which was common in the inconstant group

Table 5 shows that loud murmurs e g grade 3 or 4, were significantly ($p < 0.01$) more commonly permanent than fainter murmurs

Transient and inconstant murmurs were characterized by being more of the decrescendo type fainter and transmitted to the left sternal border and of medium frequency

Patients who died in hospital all (with one exception) had pansystolic usually high-frequency and rather loud murmurs (8 out of 19 grade 3 or 4) without total papillary muscle rupture

4. VARIABILITY OF MURMUR CHARACTERISTICS DURING HOSPITALIZATION

The murmur that appeared was not constant in character but showed great variability in type and intensity during the course of observation

Change of type (Table 6) The incidence of variation in type was high Of the murmurs in 117 patients with MIMI only 67 (57 %) were permanently of the same type The ejection type was the most

Table 6 *Variation types of murmur*

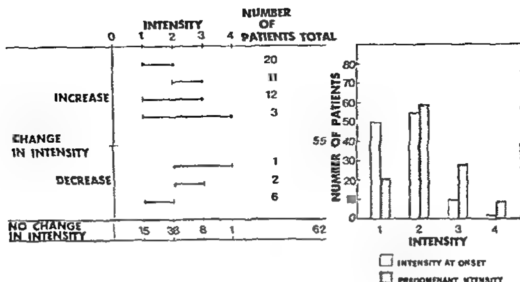
		VARIATION TYPES OF MURMUR									
		Pansystolic				*Protomesosystolic*		*Ejection*			
		Plateau	Crescendo	Decrescendo	Crescendo-decrescendo	Decrescendo	Crescendo-decrescendo	Early	Late	Total number of murmurs which presented variation in type	Total
PREVALENT TYPE OF MURMUR	*Pansystolic*										
	Plateau	(43)	6	5	1	2	4	6		24	67
	Crescendo	4	(6)					1		5	11
	Decrescendo	4	1	(12)		1		5		11	23
	Crescendo-decrescendo	2	1		(2)			1		4	[illegible]
	Protomesosystolic										
	Decrescendo					(0)				0	0
	Crescendo-decrescendo	1					(1)	1		2	3
	Ejection										
	Early	1		2				(2)	1	4	6
	Late								(1)	0	1
Total number of variation types of murmur		12	8	7	1	3	4	14	1	50	117

common (28 %) as a variant type, i e the type from which or into which the prevalent type changed (see also *Appendix*).

A phenomenon often noted was that the first transient murmur appearing in the inconstant murmur group was of the ejection (or protomesosystolic) type and that with the development of a constant murmur the type had changed to pansystolic. This was observed in 8 of the 27 patients (30 %) in the inconstant group (Fig 7). In the total material the ejection-type or protomesosystolic murmur of 12 patients became pansystolic — thus in addition to the 8 in the inconstant group in 4 patients it occurred without intermittent disappearance of the murmur — while the reverse occurred in only 2 patients. The type of constant murmur changed less frequently (39 %) than did the type of the inconstant or transient murmur (50 %), although the difference was not significant statistically.

Change of intensity (Figs 16 and 17). The intensity of the murmur changed almost as often (47 %) as it remained the same, and then generally to a low intensity (Fig 16). A common occurrence was the gradual increase in loudness of the murmur within a few days (Fig 17). Fig 16 shows the profile of incidence of the different murmur intensities when first noticed in comparison with the prevalent intensity. The frequency of occurrence of faint murmurs decreased and that of loud murmurs increased markedly. In many instances the increase was quite distinct, e g from grade 1 to 3 or to 4, as was observed in 15 patients. After the prevalent intensity had been established, a decrease in intensity was observed in 9 patients and only once by more than one grade (excluding transient murmurs, Fig 7).

Change of main location was very infre



F g 16 *Changes in the intens ty of the m tral systol c murmur in ho p tal*

quent The apical murmur shifted in only 3 patients to the left sternal border and conversely a murmur located in the left sternal border shifted to the apex in 5 patients In most instances the ejection type murmurs became pansystolic simul taneously (Fig 7)

Change of frequency was hardly ever found When it did occur it was also usually connected with a change of type pansystolic and high frequency and ejec tion type and medium frequency were mutually connected (Fig 15)

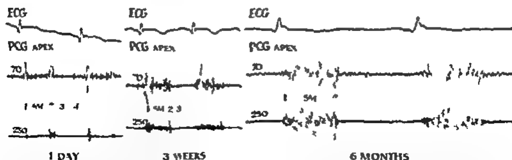


Fig 17 *Phonocard ograph c tracings showing the gradual change of the character of the m tral systol c murmur which developed 7 days after est mated onset of the myocard al infarction When appearing the murmur was grade 1—2 and of med um frequency The murmur gradually gained in loudness to grade 4 and changed predom nantly to a h gh-p tched wh le the plateau type changed to crescendo-decrescendo*

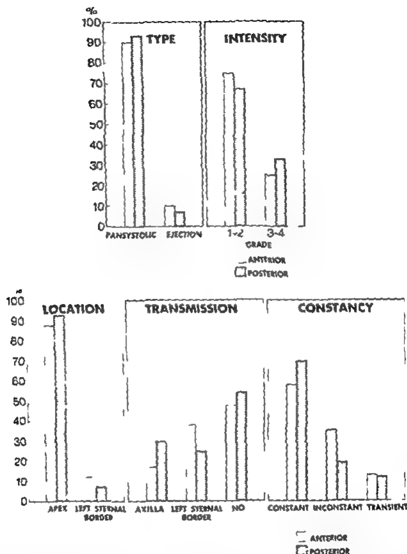


Fig 18 A comparison of the murmur characteristics in patients with anterior or posterior myocardial infarction

3 MURMUR OF ACUTE MITRAL INCOMPETENCE IN ANTERIOR AND POSTERIOR MYOCARDIAL INFARCTION

Extent of infarction and type of murmur In the MIMI group there were relatively more transmural infarctions in a posterior location (56 %) than in an anterior location (26 %)

The plateau type murmur was significantly ($p < 0.01$) associated with transmural infarction as compared with subtransmural extent Crescendo type occurred with one exception always with transmural infarction 8 of a total of 23 pansystolic decrescendo murmurs occurred in association with subtransmural infarction

Characteristics of murmur (Fig 18) The following characteristics of an acute mitral systolic murmur appeared to have a ten-

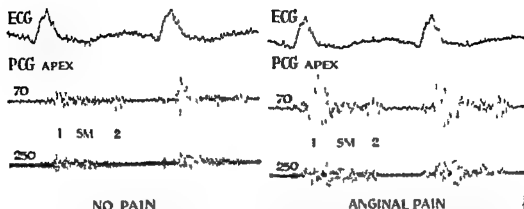


Fig 19 *Phonocardiograms confirming the transient increase in the intensity of the mitral systolic murmur during a spontaneous attack of angina pectoris The even plateau type was changed to a more uneven character The heart rate and blood pressure were not altered*

dency to be connected with the site of the infarction although the differences could not be statistically demonstrated

Anterior infarction, more often subendocardial decrescendo or ejection type or protomesosystolic inconstant, faint, located in or transmitted to the left sternal border

Posterior infarction, more often trans mural pansystolic plateau-crescendo, constant, loud, apical, and transmission to axilla

6 TRANSIENT CHANGES IN CHARACTER OF MITRAL SYSTOLIC MURMURS DURING SPONTANEOUS ANGINAL PAIN (Table 7 Fig 19)

In 8 patients with acute myocardial infarction an opportunity was afforded to observe transient and, with the relief of the pain, reversible auscultatory changes in murmurs associated with spontaneous

Table 7 *Transient changes in murmur of mitral incompetence during attacks of severe anginal pain*

Case number	Type	Intensity	Frequency	Changes in blood pressure (B P) and heart rate (H R.)
	No pain—Pain	No pain —Pain	No pain—Pain	No pain—Pain
37	*Protomeso —Plateau	2—3 — 3	Medium —High	B P 160/110 —110/—
7	— —Plateau	0 — 2	— —High	H R 90 —100
104	*Plateau —Crescendo	2 — 3	Medium—High—High	— — —
95	Plateau —Crescendo	3 — 4	(High —High)	H R 55 — 95
34	(Decrescendo—Decrescendo)	0—1 — 2	Medium—High—High	— — —
94	(Plateau —Plateau)	0—1 — 2	Medium—High—High	B P 115 65 —100/—
II	(Plateau —Plateau)	3 — 4	(High —High)	— — —
87	(Decrescendo—Decrescendo)	1 — 2	(High —High)	— — —
Main change during pain	Mainly plateau or crescendo	Always louder by 1 or 2 grades	Always high frequency	No constant change

* Several observations

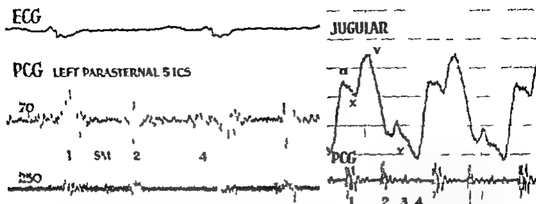


Fig 20 *Phonocardiogram of a high frequency pansystolic murmur of tricuspid incompetence which devel oped on the 8th day after the onset of the myocardial infarction The murmur was of grade 1—2 in expiration increasing to grade 3 in inspiration A marked positive jugular venous pulse appeared simultaneously with the murmur Hepatic pulsation was present*

severe anginal pain The trend during the pain was mostly to the plateau or crescendo type of previously absent, pan systolic or protomesosystolic murmur to an increase of murmur intensity by 1 or 2 grades and always a change to high frequency No constant changes in blood pressure were found a tendency to a faster heart rate during the attack of pain was noted

7 TRICUSPID INCOMPETENCE

On the 8th day after the onset of infarction one patient (No 180) developed a characteristic pansystolic murmur of isolated tricuspid incompetence in the lower sternal area with an increase of murmur intensity from grade 1 or 2 in expiration to grade 3 in inspiration A positive jugular venous pulse (Fig 20) and a hepatic pulsation developed at the same time No apical murmur was present

8 CHARACTER OF MITRAL SYSTOLIC MURMUR CAUSED BY EARLIER MYOCARDIAL INFARCTION (GROUP 3) OR IN CONNECTION WITH "CORONARY INSUFFICIENCY

Previous MI MI The murmur in patients of group 3 not included in the detailed analysis) had the following features inten sity of grade 1 to 2 always apical, pre dominant transmission to axilla high fre quency and naturally of constant oc currence Changes of type were ob served in 2 patients

Coronary insufficiency Alongside the research material consisting of infraction patients proper the author kept under prolonged observation 7 patients who were classified as belonging to the coro nary insufficiency" group (see *Methods*) These 7 patients developed the murmur indicative of mitral incompetence 1—5 days after the onset of an acute attack It was invariably pansystolic grade 2 or 3, apical, and transmitted to the left sternal border if transmission occurred (4 pa tients) The murmur was always high frequency and did not change in type from its pansystolic plateau (once a dec rescendo type was noted) In all the pa tients it eventually remained permanent after a period of inconstancy at first in 2 of them

DISCUSSION

Two of the most impressive findings of this study concerning the characteristics of the murmur of mitral incompetence as a complication of acute myocardial infarction would seem to be its great prevalence and its marked variability and dynamic features.

Incidence: No prospective systematic studies of the incidence of the mitral systolic murmur as a sequela of acute myocardial infarction are available. Most of the earlier reports, dealing with small series (see Chapter II) used the retrospective approach and seem to consist of patients with rather marked hemodynamic changes. The report published by Froment *et al.* (1955) however points to a high frequency of this complication of acute myocardial infarction in their clinical material. The present results revealed a prevalence comparable to that reported as early as 1940 by Grotel in his work on acute myocardial infarction.

This high prevalence of mitral incompetence is not, however, as surprising as the lack of clinical studies might indicate and is to be anticipated when considered in the light of autopsy data (Arkhangelskij 1959) and experimental works (Bailas 1965, Hider *et al.* 1965) as well as of acute hemodynamic alterations of myocardial infarction (see Chapter XI). It is quite clear that this high incidence of findings would not have been possible without particular searching for murmurs by meticulous auscultation. Predominantly, the murmurs were faint, in addition to which the circumstances of pursuing the study in the hospital wards included disturbing noises.

The murmur observed in the majority of the patients was typical and diagnostic of mitral incompetence (Mounsey and Bridgen 1954, Leatham 1958 a, b, McKusick 1958, Ongley *et al.* 1960, Ravin 1967), and, furthermore, a shorter or longer totally silent period before the appearance of the murmur cancels out most causes other than mitral incompetence. The harsh, atypical ejection type murmur of mitral incompetence at the apex or the left sternal border may be confused with the murmur of aortic sclerosis (Mounsey 1957, Bruns and van der Hauwaert 1958, Schimert *et al.* 1960, Spencer and Greiss 1962). However, this type of mitral systolic murmur was rather rare.

An uncertain factor of greater significance in evaluating the development of the murmur of mitral incompetence would be present if a previously present pansystolic murmur of mitral incompetence would disappear under the initial shock, as may occur in a case of ejection type systolic murmur of aortic origin. This does not, however, occur, although the intensity most often quietens down for hemodynamic reasons (Braunwald *et al.* 1957, 1958). The persistence of the murmur is related to the persistence of the clear systolic pressure gradient on the level of the atrioventricular valve causing the regurgitant flow, notwithstanding the marked decrease in blood pressure (Hultgren and Leo 1959, Lewis 1962, Harrison and Dexter 1963).

In the present series, the intensity of the murmur often simultaneously increased despite a marked decrease in the systolic blood pressure. This stresses the dominance of the mechanism of ischemic damage over the hemodynamic factors affecting the regurgitation.

Intensity: Special emphasis must be laid on the low intensity of the murmur of mitral incompetence following acute myocardial infarction.

It may be asked whether the observation of a faint, grade 1 or 2 mitral systolic murmur has any significance. The fact is that such a faint murmur is often apt to be the only sign (Nixon and Wooler 1963 b, Leighton *et al.* 1966) of the added burden inflicted on an already damaged heart by the useless flow of blood back and forth resulting from a mitral valvular

leak. This can lead to irreversibly progressive left ventricular failure (see Chapter VII).

The intensity of a murmur does not necessarily have any correlation to the severity of the mitral incompetence; it may even be lacking in hemodynamically very severe mitral incompetence, known as "silent mitral incompetence" (Logan and Turner 1952, Bridgen and Leatham 1953, McDonald *et al.* 1957, Morais *et al.* 1957, Schrire *et al.* 1961, Logan *et al.* 1967). This is obvious enough, as the velocity of the regurgitant jet, rather than a large volume flow, is the most important determinant of murmur intensity (Bruns 1959).

This lack of correlation between murmur intensity and the clinical or hemodynamic evaluation of the severity of MIMI was also clearly evident in the present investigation. A murmur intensity of grade 1 or 2 was observed in connection with marked regurgitation (e.g. cases 67 and 84, *Appendix*, and Chapter VIII).

The low intensity of the murmur and also its only gradual gain in loudness from very faint to, in some instances, fairly loud, as also noted by Nezlin and Shamesova (1951), are contrary to the loud, sudden, harsh murmur caused by avulsion of a papillary muscle.

The low intensity of the mitral systolic murmur is obviously related to the predominantly somewhat slight regurgitation and decreased contractile velocity and power of the left ventricle observed in ischemic damage (Braunwald *et al.* 1957, Ross *et al.* 1960, Rushmer *et al.* 1963).

The time of onset of the murmur was distributed over a fairly wide range. A murmur was by no means an immediate manifestation of acute myocardial infarction. The most important portion appeared during the first 5 days; this is consistent with the times of onset reported by other investigators (Grotel 1940, Nezlin and Shamesova 1951, Gorlin 1966). The somewhat delayed onset of MIMI seems to coincide with the most extensive necrotic changes in the myocardium, which take place a few days after the onset of the ischemic damage (Edmondson and Hoxie 1942, Lodge Patch 1951, Bargmann and Doerr 1963). A similar timetable is also to be noted in the major destructive complications discussed earlier, e.g. ventricular septum perforation, papillary muscle disruption and myocardial rupture.

The *type* of the mitral systolic murmur was predominantly pansystolic, characteristic of mitral incompetence, which means regurgitation throughout systole. The pansystolic type of murmur was also observed by the majority of other investigators (Grotel 1940, Nezlin and Shamesova 1951, Orlando *et al.* 1964, Bashour 1965, Raftery *et al.* 1966, Soulie *et al.* 1966).

The murmur of papillary muscle dysfunction described by Burch *et al.* (1963) and Phillips *et al.* (1963 a) was of the clear cut ejection type, but most of their patients seemed to be suffering from chronic ischemic heart disease, only a few patients having had acute myocardial infarction.

Ejection type murmurs similar to the diamond shaped murmur described by these writers occurred in the present study in many instances during the very first few days of infarction. Later often after a temporary disappearance, the murmur definitely changed in type to pansystolic. The first phase of the regurgitation might be related to the circumstance of a papillary muscle being rendered incapable of shortening during the ejection phase as a consequence of ischemia, although still able to generate tension during the initial isovolumetric phase of the systole (Burch *et al.* 1963). This mechanism obviously causes an ejection-type murmur. In the second phase the total loss of contractile power caused by extensive necrotic destruction of papillary

muscle would allow the development of a pansystolic type murmur (For further mechanisms of regurgitation, see Chapter XI)

The decrescendo type of murmur often seemed, like the ejection type, to indicate a mild degree of mitral incompetence when judged by the character of the murmur The decrescendo type of murmur — pansystolic or protomesosystolic — has been recognized as usually indicating only insignificant mitral incompetence (Gorlin *et al* 1952 Hultgren and Leo 1959, Nager *et al* 1964 Holldack and Wolf 1966, Ilmurzynska 1966) Otherwise it was the crescendo type of murmur that was most frequently observed in association with marked regurgitation (See also the section on murmur types at follow up Chapter X)

Transient murmur changes during intense anginal pain, without consistent changes in heart rate or blood pressure would further support the view that a mechanism of ischemic malfunction of the papillary muscle operates in murmur generation

The recent view that the late systolic murmur is usually related to disease of the chordal papillary muscle apparatus (Humphries 1964, Segal and Likoff 1964 Phillips *et al* 1963 a Barlow and Bosman 1966 Criley *et al* 1966) is consistent with the observations of its occurrence in the present study

Site of infarction The auscultatory characteristics of murmurs suggest that damage to the posterior papillary muscle is more serious than damage to the anterior papillary muscle This suggestion is consistent with the higher incidence of involvement of the posterior papillary muscle in the autopsy findings in the present as well as earlier studies (Nezlin and Shamesova 1951 Orlando *et al.* 1964, Bashour 1965)

The direction of radiation of the murmur in anterior and posterior myocardial infarction was different from that generally described in patients with chorda tendinae ruptures or rheumatic valvular disease. The murmur in posterior infarctions radiated predominantly to the axilla in the present study and not to the aortic area (Edwards and Burchell 1958, Osmundson *et al* 1961, Sleeper *et al* 1963, Burchell 1963, Menges *et al* 1964 Raftery *et al* 1966) This might be ascribed to louder intensity of the murmur in the cases described On the other hand the mitral systolic murmur associated with anterior infarction had more of a tendency to radiate to the aortic area, as in cases with anterior chordal rupture (Shapiro and Weiss 1959 Miller and Pearson 1959) and as in anterior papillary muscle dysfunction (Phillips *et al* 1963 a) The transmission of faint murmurs may be easier to the left sternal border than to the axilla, where the lung a poor sound conductor, interposes (Leighton *et al* 1966)

To sum up, the high prevalence of mitral incompetence and the marked variability of the intensity and type of murmur, together with its frequently transient and inconstant character may be directly related to the basic background mechanism, an ischemic myocardial injury, which often varies in extent and severity from moment to moment The contractile function and size of the left ventricle, and the function of the papillary muscles in their various phases of contraction, are susceptible to ischemic impairment in varying degrees This explains the highly variable auscultatory characteristics of mitral incompetence caused by acute myocardial infarction.

COMPARISON OF CLINICAL FINDINGS IN PATIENTS WITH AND WITHOUT MITRAL INCOMPETENCE

CLINICAL FINDINGS

PREDISPOSING FACTORS IN THE DEVELOPMENT OF MITRAL INCOMPETENCE

PREVIOUS CORONARY HEART DISEASE

The patients' previous symptoms of coronary heart disease and the occurrence of certain other diseases of the cardio vascular system were studied in order to find possible predisposing factors in the development of mitral incompetence In this respect, as in the later comparisons, only groups 1 and 2, consisting of 187 patients, were compared for the reasons given in the foregoing (see Chapter V)

Angina pectoris No differences could be detected between the MIMI and no-murmur groups in the occurrence or duration of earlier angina pectoris Angina pectoris had been experienced by slightly more than half the subjects, 58 % in the MIMI and 54 % in the no murmur group

Reinfarction A previous attack of infarction appeared to make a patient more susceptible to the development of a mitral incompetence in connection with reinfarction 72 % of the MIMI patients and 84 % of the no-murmur patients had first infarctions, the reinfarction figures being 28 % and 16 % respectively However, the difference was not significant The total number of patients with reinfarctions was 44 out of 187 patients (24 %)

OTHER DISEASES OF THE CARDIOVASCULAR SYSTEM

The occurrences of hypertension, congestive heart failure, intermittent claudication and/or signs of peripheral arterial disease, cerebrovascular accidents and diabetes mellitus were analyzed

Major peripheral arterial disease manifestations, such as intermittent claudication, or murmurs or absent pulsations in large peripheral arteries (17 %) or cerebrovascular accidents (5 %), did not differ in relative incidence in either group of patients These diseases affected 22 % of the patients

There were 11 patients with *diabetes*, of whom 9 belonged to the MIMI group Only definite, manifest diabetes was included in the analysis, and not prediabetes or, of course, transient glucosuria observed on its own in association with infarction, since the material in this respect had been non uniformly studied The difference was not, however, statistically significant

Heart failure of N Y H A grade II or more in the patients history of subjective symptoms was found in 16 patients in the MIMI and 6 in the no-murmur group, with no significant difference

Hypertension was found to have a significantly ($p<0\ 01$) higher incidence among MIMI patients (33 %) than among those in the no murmur group (14 %) On the other hand, there was no significant difference within the groups as regards systolic or diastolic hypertension and the distribution of their degree of severity Hypertension was present in 49 of the total of 187 patients (26 %)

SEVERITY OF ACUTE MYOCARDIAL INFARCTION
(Fig 21)

The severity of myocardial infarction was estimated on the basis of the coronary prognostic index developed by Peel *et al* (1962) This estimation made upon the patient's admission to hospital, takes into account in part the afore mentioned factors from his medical history and in addition his initial clinical situation The distribution in the different severity groups was remarkably uniform among both MIMI and no-murmur patients (Fig 21)

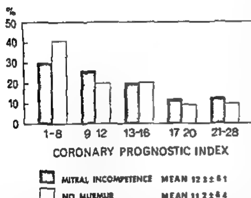


Fig 21 *Distribution of the coronary prognostic index in patients with and without mitral incompetence No difference was evident between the patient groups*

The laboratory tests yielded the following average results in the MIMI and the no-murmur groups of patients The average maximal erytrocyte sedimentation rates were 51 mm/hour in the MIMI group and 44 in the no-murmur group The maximal white cell counts were 15,000 and 11 800 in mm³, and the maximal serum GOT in the MIMI group was 228 and in the no-murmur group 124 units

CLINICAL SEQUELAE OF MITRAL INCOMPETENCE

Degree of mitral incompetence As a background for the findings to be described in the following, together with their possible connection with mitral valvular incompetence, Table [illegible] presents the severity, as judged clinically, of mitral incompetence following myocardial infarction However, it must be pointed out that in many instances the easy estimation of the hemodynamic severity of mitral incompetence becomes an exceptionally difficult task when mitral incompetence is superimposed on acute myocardial infarction

The generally used clinical signs indicating severe mitral incompetence with reasonable accuracy are sustained and hyperdynamic apical impulse soft first heart sound, prominent ventricular gallop and diastolic flow murmur, loud pansystolic apical murmur transmitted to the axilla low arterial pulse pressure splitting of second heart sound and an increase in its pulmonary component and electrocardiographic signs of left ventricular and atrial hypertrophy (Bridgen and Leatham 1953 Wood 1954 Harvey *et al* 1957, McKusick 1958 Hubbard *et al* 1959 Bentivoglio *et al* 1961, Perloff and Harvey 1962 Nixon 1961 1963, Nixon and Wooler 1963 a, b, Humphries 1964 Deliyannis *et al* 1964) However, most of these findings also occur in acute myocardial infarction A pansystolic apical murmur is a hallmark of mitral incompetence, and though a loud murmur usually indicates a large regurgitation (Gorlin *et al* 1952 Wade *et al* 1952, Abelmann *et al* 1953), bad mistakes can happen if its intensity is correlated directly to the amount of regurgitation (see Chapter VI) What actually remains is only a hyperdynamic left ventricular impulse

Table [illegible] presents the assessment of marked mitral incompetence to be present in 20 patients which is 17 % of the whole group Thus, hemodynamically, regurgi-

Table 8 *Severity of mitral incompetence evaluated by clinical judgment and its relation to acute mortality*

Degree of MMI	Number of patients	Died in hospital
Marked	20 (17 %)	8 (40 % of 20)
Slight	97 (83 %)	13 (12 % of 97)
Total	117	21

Table 9 *Incidence and severity of cardiogenic shock*

Degree of shock	Mitral incompetence			No murmur		
None	82 (70 %)			49 (70 %)		
Mild (score 1)	7 (6 %)		(30 %)	4 (6 %)		(30 %)
Moderate (score 5)	17 (15 %)	(24 %)		8 (11 %)	(24 %)	
Severe (score 7)	11 (9 %)			9 (13 %)		

Table 10 *Incidence of congestive heart failure at hospital*

Heart failure		Mitral incompetence		No murmur	
Acute	left	57* constant 25, transient 32*	76* (65 %)	25 constant 8, transient 17	34 (49 %)
	left and right	15 constant 8, transient 7		6 constant 3, transient 3	
	right	4 constant 1, transient 3		3 constant 1, transient 2	
Delayed	left	22*	23* (20 %)	5	5 (7 %)
	left and right	0		0	
	right	1		0	
Total number of patients with heart failure		89 (76 %)		39 (56 %)	
No heart failure		28 (24 %)		31 (44 %)	
Total		117		70	

*Includes 10 patients with acute transient failure who later developed delayed failure

tation appeared to be somewhat on the insignificant side in the majority of the patients On the other hand, the great significance of the severe mitral incompetence is demonstrated by the exceedingly high mortality in this group, 40 %, as compared to the corresponding mortality figure for the slight incompetence group, 12 %

Cardiogenic shock (Table 9) Both the incidence and the severity of cardiogenic shock were identical in patients with and without mitral incompetence developing after myocardial infarction Actual shock (scores 5 and 7) occurred in 24 % in each group Mild shock, with a score of 1, which occurred in 6 % in both groups, is a vague concept It has been included in the table because it was used in determining the coronary prognostic index, though strictly speaking (see *Methods*, Chapter IV) its inclusion within the concept of shock is open to dispute Shock appeared almost invariably on the first or the second day after onset of infarction and before the murmur of mitral incompetence was observed, in only two instances were shock and murmur found to occur in a patient simultaneously Accordingly, shock neither predisposes a patient to MIMI nor is a consequence of it Terminal shock observed in dying

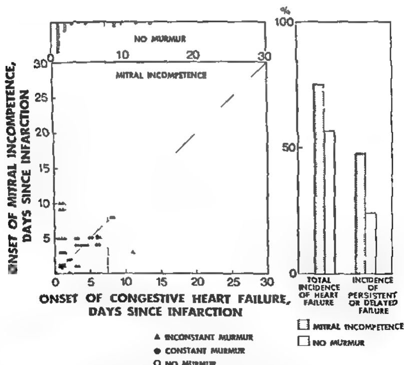


Fig 22 *Relation of the time of onset of the clinical signs of congestive heart failure to the appearance time of the mitral systolic murmur If the first day is excluded (first dotted line) the heart failure seems predominantly to follow MIMI Incidence of the persistent or delayed heart failure (appearing in the second week or later second dotted line) was significantly ($p < 0.01$) higher in the MIMI patients than in the patients without mitral systolic murmur in whom the heat failure generally appeared during the two first days*

patients, was not included in the analysis

Congestive heart failure (Table 10, Fig 22) Congestive heart failure was classified arbitrarily for detailed consideration as acute and as delayed failure, with the latter being regarded as starting during the second week or later Acute failure was divided into two categories — transient when it disappeared within a few days, and permanent when it persisted beyond a period of 16 days

Signs of congestive heart failure were observed quite frequently occurring in 76 % of the MIMI patients and 56 % of those in the no murmur group (Table10) The heart failure was predominantly left sided Acute development of heart failure was observed in two-thirds (65 %) of the MIMI group and in half (49 %) of the no-murmur group a difference which was significant at the level of $p < 0\ 02$ The groups differ more significantly ($p < 0\ 01$) from each other if one considers only the acute constant or delayed left ventricular failure combined, whereupon the percentages were 48 and 24 (Fig 22)

The no-murmur group was otherwise characterized by acute often only transient, left-sided heart failure, while what was typical of the MIMI group was the continuation of acute left sided heart failure and the frequent occurrence of delayed failure This situation is shown in Fig 22, where the time of onset of heart failure or the rapid deterioration of an existing failure has been plotted against the time of onset of mitral incompetence The heart failures present on admission, 15 patients in the MIMI and 14 in the no murmur group, have been excluded in order to obtain more valid data The failures in the no murmur group appeared in 13 of the 25 patients on the first day of illness If the first day failures are left out of the MIMI group, the signs of heart failure seemed to appear or to worsen rapidly in fairly close connection with the appearance of mitral incompetence or thereafter Delayed failure (20 %) appeared without exception in this way (e g , following MIMI), and in the no murmur group there were only 5 instances (7 %) of delayed appearance of left ventricular failure ($p < 0\ 05$)

The use of digitalis gives some indication of the presence of heart failure, and

Table 11 *Relation of severe pulmonary edema to acute mitral incompetence*

Time relations	Number of patients	Onset of pulmonary edema days after infarction	Hypertension	Pressor amine used	Died	Recovered
Pulmonary edema before mitral incompetence	6 (−1)*	1 ± 0	3	3	1	[illegible]
Mitral incompetence before pulmonary edema	8**	4 1 ± 3 2	6	3	7	1
Pulmonary edema in patients without mitral incompetence	5	1 ± 0	1	1	1	4
Total	19		10	7	9	10

*One patient had initial but transient pulmonary edema and after development of MIMI a recurrence occurred which proved fatal

**In 7 patients the marked mitral incompetence was probably the major cause of the pulmonary edema

Table 12 *Patients with pulmonary edema following acute mitral incompetence*

Case number	Duration of MIMI before onset of the pulmonary edema days	Duration of the pulmonary edema days	Fate of patient	Hyper tension	Use of pressor amine
99	4	1	Died	−	−
4	3	3	Died	+	+
5	3	3	Died	+	+
2	1	3	Died	−	−
17	1	3	Died	−	+
8	½	4	Died	+	−
117	½	2	Recovered	+	−
12	simultaneous	6 hours	Died	+	−

although the criteria obviously vary, they were nevertheless the same for both groups of subjects Digitalis was used more commonly in the MIMI group (68 %) than in the no-murmur group (40 %)

Acute pulmonary edema (Tables 11 and 12) Acute pulmonary edema often appeared on the very first day of illness, as did shock or less severe signs of left ventricular failure A total of 19 patients (10 %) suffered pulmonary edema Of these in 6 in the MIMI group and in all 5 in the no murmur group the edema developed during the first day of illness

All 8 patients who only later developed pulmonary edema were from the MIMI group and in 7 of these patients the presence of acutely developed mitral incompetence was regarded on clinical grounds as definitely contributing to the development of pulmonary edema In these patients the degree of MIMI was already clinically estimated as marked before the pulmonary edema developed Table 11 presents the relation of pulmonary edema to mitral incompetence When the pulmonary edema appeared before the MIMI, its duration was only 1—2 days, with a good response to treatment

Otherwise the pulmonary edema appearing after (average 4 1 ± 3 2 days after onset of infarction) the development of MIMI (Table 12) was resistant to treatment and the majority of the patients died the difference in mortality being significant ($p < 0\ 01$) when compared with the other two groups

The part played by hypertension in the development of pulmonary edema in patients with mitral incompetence seemed apparent, 9 of the 39 in this group with hypertension having developed pulmonary edema, whereas only 1 patient of the 10 with hypertension in the no-murmur group was so affected The difference was not, however, statistically significant The use of pressor amines to elevate lowered blood pressure in connection with pulmonary edema after the development of mitral incompetence would seem to have contributed to increased mortality, although the small number of patients involved made statistical analysis impossible

Blood pressure did not differ significantly between patient groups in initial or stabilized phases at hospital In MIMI group the values of systolic pressure were 140 ± 30 mm Hg in the initial phase before development of the murmur and 138 ± 23 in the stabilized phase 3—4 weeks from onset In the no-murmur group the respective figures were 132 ± 22 and 130 ± 25 mm Hg

Table 13 *Arrhythmias during stay in hospital*

Arrhythmia	Mitral incompetence	No murmur	Total
Total number of patients	117	70	187
None	37 (32 %)	35 (50 %)	72 (39 %)
Sinus tachycardia	27* (23 %)	7** (10 %)	34 (18 %)
Premature beats	47 (40 %)	21 (30 %)	[illegible] (36 %)
Supraventricular tachycardia	2 (2 %)	2 (3 %)	4 (2 %)
Atrial fibrillation or flutter	8 (7 %)	5 (7 %)	13 (7 %)
Nodal rhythm	1 (1 %)	1 (1 %)	2 (1 %)
Heart block	12 (10 %)	5 (7 %)	19 (9 %)
First degree	2 (2 %)	2 (3 %)	4 (2 %)
Second degree	6 (5 %)	2 (3 %)	8 (4 %)
Complete	4 (3 %)	1 (1 %)	5 (3 %)

* 15 died ** 1 died

Arrhythmias (Table 13) were observed in daily clinical examination and routine electrocardiograms in 62 % of the patients. They were significantly ($p<0.01$) more frequent in the MIMI than the no-murmur group. Sinus tachycardia was highly significantly ($p<0.001$) connected with MIMI and with acute mortality. Complete heart block developed in 4 patients in the MIMI and 1 in the no-murmur group. These were treated successfully by transvenous endocardial pacing until the sinus rhythm returned. One patient (No. 124) developed a permanent complete heart block, requiring the application of an implantable pacemaker.

No difference in incidence or character of the *apical impulse* was found when comparing the MIMI and no-murmur groups. It was not palpable in 44 % of both groups. Heaving apical thrust was present in 8 patients with *mitral incompetence* and 7 without mitral systolic murmur. A location at a distance of 10 cm or more from the midline was found in 40 % and 37 %, respectively.

Paradoxical cardiac pulsation (Figs. 23 and 24) could be recognized frequently at

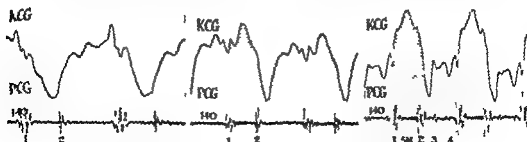


Fig. 23. *Three kinetocardiographic tracings (KCG) with phonocardiographic references to illustrate the phenomen of the paradoxical cardiac pulsation. The first curve reveals the normal inward retraction of the apical impulse after an initial brief outward thrust. The second tracing shows a moderate systolic outward bulge (stippled area) lasting not over the whole systole. The third record represents a marked pansystolic bulge*

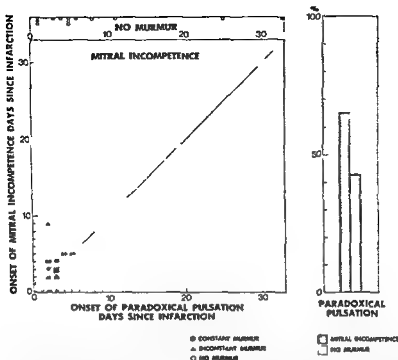


Fig 24 *Relation of the paradoxical cardiac pulsation to the appearance of the mitral systolic murmur Paradoxical pulsation was a frequent bedside finding (51%) and developed significantly more commonly in the MIMI group Its onset time was within ±1 day (dotted lines) the same as the onset of the murmur in a half of the patients*

bedside examination the total incidence of this palpatory finding was 51% It was present significantly ($p<0.01$) more commonly in the MIMI group (65%) than in the no murmur group (43%) During the course of hospital observation it developed as a new finding in 49 (42%) MIMI patients and 15 (21%) no-murmur patients this difference being also significant ($p<0.01$) The bulge was moderate in one third of the patients and marked in two thirds of both groups Occurrence with constant mitral systolic murmurs was more frequent (74%) than with only inconstant or transient murmurs (60%)

In Fig 24 the time of onset of the paradoxical pulsation has been plotted against the time of onset of mitral incompetence in the 49 patients in whom both developed during the observation period (the 27 MIMI patients with paradoxical pulsation on admission are excluded) Mitral systolic murmur and paradoxical pulsation appeared on the same day with a variation of ±1 day in half of the patients or in 24 out of 49 In 40 patients a kinetocardiographic analysis of precordial pulsation showed a close agreement with the palpation finding

Changes in first and second heart sounds (Table 14) Softening of first sound fainter than second sound at the apex was observed in 19% of the MIMI group and in 3% of the no-murmur group or in a total of 24 patients The pulmonary component of second heart sound was estimated to be accentuated in 39 patients This was noted in 27% of the MIMI and 10% of the no-murmur patients

Table 14 *Changes in first and second heart sounds*

	Mitral incompetence	No murmur	P
Total number of patients	*117*	*70*	
1st sound diminished (1/2 at apex)	22 (19 %)	2 (3 %)	<0 01
Pulmonary component of the 2nd sound accentuated	32 (27 %)	7 (10 %)	<0 01
Pulmonary component of the 2nd sound accentuated with broad splitting of the 2 nd sound	20 (17 %)	1 (1 %)	<0 01
2nd sound broadly splitting but pulmonary component not accentuated	0	0	

Among these patients with an accentuated pulmonary component, a broader than usual physiological splitting of second heart sound during inspiration was found in 63 % of the patients in the MIMI and in 14 % of the no-murmur group All these differences were significant ($p < 0.01$) Curiously enough, pathologic splitting without simultaneous increase in loudness of the pulmonary component of second heart sound was never observed The changes developed in about half the patients after development of MIMI, while in about half they were already present on admission In the no-murmur group the changes were usually present on ad-

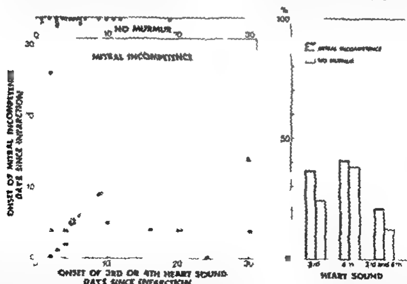


Fig 25 *Relation of the time of onset of third and fourth heart sounds to the onset of mitral incompetence and their incidence Third heart sound developed chiefly after the appearance of the mitral systolic murmur The appearance of the atrial gallop sound was early without clear relation* ■ *the MIMI* ● *3rd sound constant MIMI* ○ *3rd sound inconstant MIMI* ▲ *4th sound constant MIMI* △ *4th sound inconstant MIMI* ◗ *3rd sound no murmur* ○ *4th sound no murmur*

mission, with their development during the observation period taking place in only an occasional instance

Occurrence of third or fourth heart sounds (Fig 25) was observed by auscultation in 57 % of the patients in groups 1 and 2, in 62 % of the MIMI patients and in 47 % of the no-murmur patients

Third heart sound was recognized transiently or constantly in 60 (32 %) patients, occurring in the patients with MIMI in 38 % and in the patients without mitral systolic murmur in 23 %, the significance of the difference was at the level of $p<0.05$ If only newly-developed ventricular gallops are considered excluding those patients with the gallop present on admission, the figures were total 23 %, MIMI 28 % and no-murmur group 14 % Groups 1 and 2 differed significantly at a level of $p<0.05$

Fourth heart sound was heard in 73 patients (39 %), in the MIMI group in 41 % (newly developing 33 %) and in the no-murmur group in 36 % (newly developing 25 %), with no significant statistical difference

Both third and fourth heart sounds in the same patient occurred in 20 % in the MIMI and 13 % in the no-murmur group

As previously noted (Chapter VI) third and fourth heart sounds were not over diagnosed, but 2 % of third and 10 % of fourth heart sounds were missed

Fig 25 relates the onset time of third and fourth heart sounds to the time of onset of the mitral systolic murmur Comparison is available from 28 MIMI patients (16 excluded because the gallop was present on admission) and 9 no-murmur patients (7 excluded) with third heart sound, and from 34 (14 excluded) MIMI patients and 15 (10 excluded) no-murmur patients with fourth heart sound

It is evident that third sound appeared mostly at or after the onset of the murmur of mitral incompetence The appearance time of third sound was thus broadly distributed in many instances it appeared rather late

Fourth sound did not seem to have any clear relation to the appearance of the mitral systolic murmur and was earlier in origin than third sound In the no-murmur group the distribution of fourth sound was likewise earlier than that of third sound However the delayed appearance of third sound was found much more commonly in the MIMI than in the no-murmur group, as was the situation with the signs of congestive heart failure

Reinfarction groups It was previously (p 50) noted that an earlier infarction seemed to have a predisposing effect on the development of mitral incompetence in connection with reinfarction Therefore, some of the most distinct changes already found in patient groups 1 and 2 were re analyzed with reference to reinfarction

The murmur characteristics did not differ clearly from those found in patients with first infarction although with a single exception the murmur was invariably pansystolic Heart failure and paradoxical pulsation did not differ from the data already gathered but hypertension was commoner in MIMI reinfarction (42 %) than MIMI first infarction (29 %) A smaller difference was found in the no-murmur group 18 % in reinfarction and 14 % in first infarction

DISCUSSION

INCIDENCE OF VARIOUS CLINICAL FINDINGS

The incidence of the various clinical bedside findings observed in the present study was generally of the same order as in many very large series (Master *et al* 1937, 1942, Rathe 1942, Mintz and Katz 1947 Yater *et al* 1948 Doscher and Poindexter 1950, Laubry and Soulie 1950, Jakobs 1951, Wright *et al* 1954 Ball *et al* 1955 Lee *et al* 1957, Plotz 1957, Jakobs 1958, Malach and Rosenberg 1958 Sievers 1963 Friedberg 1966)

Considerably more often than has been generally reported however, signs of congestive heart failure, third and fourth heart sound and paradoxical cardiac pulsation occurred The discrepancies in numbers may be attributed to the frequently very different criteria applied by different investigators, while, on the other hand special attention was paid in the frequent examinations carried out in the present study to these often transient clinical signs The incidence figures refer to groups 1 and 2 for purposes of comparison and they obviously differ to a slight extent from those calculated from the entire series

PREDISPOSING FACTORS IN MIMI

Predisposing factors in the development of mitral incompetence as a result of myocardial infarction extracted from the patients earlier medical history seemed to be hypertension, previous myocardial infarction and manifest diabetes, of these only hypertension in a significant degree Otherwise peripheral arterial disease and angina pectoris could not be proved as having such an effect

The greater tendency of *reinfarction* to be attended by the complication of mitral incompetence appears only natural, for in most instances conspicuous coronary artery changes are present while furthermore a portion of the myocardium has been previously destroyed Reinfarction thus has a directly greater chance of injuring the supporting structures of the mitral valve i e either the papillary muscles or by inducing aneurysm or general dilatation of the heart On the other hand a coronary artery occlusion may remove the barely sufficient collateral circulation from the portion of the left ventricle critically important to the closure of the mitral valve

Hypertension as a contributing factor accelerating atherosclerotic changes appears obvious on the evidence of both experimental and clinical investigations (Dawber *et al* 1957, Schettler 1961, Moses 1963) Its influence as a predisposing factor in the development of a mitral incompetence may come from this source

On the other hand, the added pressure load on the heart leads to the hypertrophy of the muscle fibres Consequently the nutrition of the myocardium is impaired considerably in response to the relative coronary arterial insufficiency (Vizell 1951, Linzbach 1960) The disturbance in microcirculation is greatest in the subendocardial portions, due to the anatomy of the coronary vessels and intravascular and intramyocardial pressure relations (Johnson and DiPalma 1939, Schutz 1956, Laszt and Muller 1958, Muller 1962, Salisbury *et al* 1963 a) In these subendocardial layers of the left ventricle, the oxygen tension is lower than in the outher parts (Moss 1966), and ischemic changes easily develop Arosemena *et al* (1966) in 85 non coronary cases of hypertrophy of the left ventricle found a significant degree of infarction of the papillary muscles and the adjacent left ventricular wall This was believed to be the result of impaired myocardial perfusion

Diabetes has evidently an adverse effect on the development of atherosclerotic lesions and furthermore may be related to microangiopathy Among diabetics the myocardial damage caused by infarction is more extensive and the disease more serious than is the general rule (Partamian and Bradley 1965)

Coronary prognostic index The very great variability known to prevail in the clinical picture of myocardial infarction has resulted in attempts to assess it by means of different clinical indices (Helander 1950 Russek *et al* 1951 Schnur 1953) The coronary prognostic index values obtained by the method of Peel *et al* (1962) correlated well with the acute mortality of the myocardial infarction patients in hospital recorded in the present study

The completely uniform distribution in the different degrees of severity of the

index used for both the MIMI and the no murmur patients indicates that the severity of myocardial infarction as clinically judged at the initial stage is no determining factor in the development of mitral incompetence This finding is further supported by the evidence of an equal incidence of initial heart failure and shock in both groups of patients The result emphasizes the significance of an infarct of perhaps small extent but strategically important location with reference to papillary muscles The even distribution of the coronary prognostic index in both groups also points to the lack of selectivity in the material from this point of view, providing a sound basis for further comparison of the findings

CLINICAL SEQUELAE OF MIMI

Cardiogenic shock The reports on the incidence of shock vary quite considerably 8—58 % which is understandable owing to the difficulty of defining the clinical conception (Master *et al* 1937, Bean 1938 Yater *et al* 1948 Wright *et al* 1954, Binder *et al* 1955, Agress and Binder 1957, Friedberg 1966) The average incidence has been regarded as involving one quarter of the patients which was also found in the present investigation (24 %) Shock was the initial disturbance in this study, in line with the general view

Hemodynamic disturbances in cardiogenic shock are marked in both coronary and systemic circulation A marked decrease in cardiac output and stroke volume, by 25—80 % is the invariable result (Wegria *et al* 1954, Gilbert *et al* 1954, Agress and Binder 1957, Wollheim and Schneider 1958, Malmcrona 1964, Gunnar *et al* 1966) Remarkably enough this profound circulatory disturbance did not have any effect, however on the development of MIMI

The decrease in coronary perfusion in shock has a damaging effect on the heart, though the lessening of the pressure load in hypotension reduces the burden of work on the heart These changes might conceivably cancel out their effects from the standpoint of the heart's susceptibility to injury Experimental support to this view is given by the investigations of Opdyke and Foreman (1947) and of Gregg (1962) on coronary circulation in shock

Congestive heart failure was a more frequent finding (68 %) in this study than has been generally reported the average incidence in previous studies being from a third to half of the patients However, Friedberg (1966) and Logue and Hurst (1966 a) emphasize that signs of left sided heart failure are often overlooked Further, recent radiological and pulmonary function studies in acute myocardial infarction indicate that pulmonary congestion and impairment of ventilatory function are very common These changes are obviously due to acute left ventricular failure (Fauda *et al* 1957, Zatuchni and Nussbaum 1963, Uthgenant 1964, McNicol *et al* 1965, Valentine *et al* 1966) The results of the radiological examinations performed in the course of the present study likewise showed a frequent occurrence of signs of pulmonary venous hypertension

The initial incidence of left ventricular failure on admission, or developing during the first day, was not higher in the MIMI group (29 %) than among no-murmur patients (39 %) Otherwise, the perpetuation and worsening of initial heart failure as well as the development of clinical signs of delayed left ventricular failure, delayed pulmonary edema and third heart sound were definitely and significantly associated with the development of mitral incompetence In contrast to the MIMI group, the heart failure in the no-murmur group was more often only initial and transient

Although in the majority of patients the mitral incompetence was clinically judged to be somewhat insignificant, it would thus seem that even a slight

added measure of futile volume work resulting from it had the effect of generating considerable functional difficulties in an acutely damaged left ventricle

Acute pulmonary edema had the same relation to MIMI as the less marked manifestations of left ventricular failure. Pulmonary edema during the first day after the onset of infarction was of equal incidence in both groups. Any subsequent development of pulmonary edema occurred in almost all the patients only after prior development of acute mitral incompetence. Such delayed pulmonary edema following MIMI was difficult to treat and usually proved fatal

Systemic arterial hypertension is a frequent contributing factor to pulmonary edema in myocardial infarction (Master *et al* 1937 Plotz 1957) and in the present study it seemed to exercise a particularly important effect if the patient had developed pulmonary edema following MIMI. *The last resort* would seem to be treatment of this grave multitude of complications with pressor amines (see also Chapter XI). It is obvious that the rising of the peripheral systemic arterial resistance with pressor amines substantially increased the regurgitant flow through the incompetent mitral valve (Wiggers and Feil 1922 Braunwald *et al* 1957 Jose *et al* 1964 Fusada 1965). In the presence of pulmonary edema in a patient with mitral incompetence following acute myocardial infarction this treatment must virtually always prove to be a fatal procedure

This serious situation could be reasonably explained by the frequent initial mechanical failure of the heart as a pump after *myocardial infarction* (Malmcrona 1964). Thus the acute additional demand of the regurgitant flow becomes intolerable. The markedly diminished contractile power of the left ventricle, combined with mitral incompetence obviously often leads to a progressive and eventually fatal vicious cycle (see also Chapter XI). Thus all therapeutic measures liable to increase regurgitation must be deemed contraindicated

The gloomy course run by a small proportion of the patients developing MIMI was comparable to the fatalities in the series of Nezlin and Shamesova (1951). Bashour (1965), Chiesa *et al* (1965) and Raftery *et al* (1966) also lay stress on the occurrence of significant heart failure

Gallop sounds were also detected fairly frequently (57 %) in auscultation, confirmed by phonocardiography, as compared with reports of their incidence varying between 5—28 % of the patients with acute myocardial infarction (Bean 1938, Master and Friedman 1942, Rathe 1942, Shillito *et al* 1942, Yater *et al* 1948, Jakobs 1951, Wright *et al* 1954, Weitzman 1955, Malach and Rosenberg 1958). In part, this can be attributed to the fact that third or fourth sound is sometimes transiently audible for only a few days. However, Master and Friedman (1942) in their *phonocardiographic investigation* revealed the presence of fourth sound in 83 % (audible gallop in 33 %) and third sound in 47 % (audible gallop in [illegible] %) in myocardial infarction

Friedberg (1966) also emphasizes that the gallop is often transiently audible at the initial stage in approximately one quarter of the patients. He further states that while third sound is generally accepted as a sign of heart failure, fourth sound is not associated with it. The results in the present study were fundamentally the same. Patients with MIMI had more often gallop sounds — the ventricular gallop being more common, significant at the level of $p<0.05$ — than did the patients *without mitral systolic murmur, in whom* these occurred transiently during the first days in hospital

Third heart sound is generally accepted as a sign of heart failure (Lewis 1962). On the other hand it is associated with significant mitral incompetence owing to the increase in rapid diastolic filling (Leatham 1958a, Nixon 1961, Nager *et al* 1964)

The final mechanism of the generation of third heart sound is still in dispute A connection with sudden tautening of the chordae tendinae (Nixon 1961, 1963 Nixon and Wooler 1963 b) and with active and passive ventricular relaxation and volume pressure accommodation has recently been suggested (Grayzel 1960, Crevasse *et al* 1962, Arevalo *et al* 1964) In any case, the ventricular gallop is a clinical sign present in diastolic overloading of the ventricle if hyperkinetic states and younger age groups are excluded (McKusick 1958)

The occurrence of third heart sound more commonly in the MIMI group, and predominatly only after the development of mitral incompetence would point to its connection with regurgitation Otherwise the occurrence and time of onset of congestive heart failure bore the same relation to MIMI Probably both these factors causing diastolic overloading of the left ventricle were operating (McKusick *et al* 1955 Parry and Mounsey 1961)

Fourth heart sound as a sign of an atrial overload (Ongley *et al* 1960) did not have so intimate a relation as third sound to the appearance of the MIMI and the higher incidence in the MIMI group was not significant Fourth heart sound can occasionally be heard in normal subjects (Segall 1962) Nevertheless, its frequent early development during the course of acute myocardial infarction would indicate strain or some other abnormality of the left ventricular myocardium (Butterworth and Reppert 1966) The mechanism of the atrial gallop in myocardial infarction has been ascribed to diminished ventricular distensibility by the ischemic damage (Weitzman 1955, Kincaid Smith and Barlow 1959, Parry and Mounsey 1961) An abnormally large atrial wave in kinetocardiography or apexcardiography is a frequent finding in an ischemic heart disease (Benchimol and Dimond 1962, Dimond and Benchimol 1963, Schweitzer *et al* 1965, Ginn *et al* 1967)

First heart sound Recent studies suggest that the most important factor affecting the intensity of first heart sound is the contractility of the myocardium (Sakamoto *et al* 1965) Accordingly, the weakening of first heart sound in myocardial infarction is natural, if not invariable (Master and Friendman 1942, Laubry and Soulie 1950) First heart sound in mitral incompetence has been variously described, its diminished intensity has usually been related to marked regurgitation (Bridgen and Leatham 1953, Wood 1954, McKusick 1958 Perloff and Harvey 1962, Nager *et al* 1964) In the light of the present study, it is significantly more frequently diminished in the MIMI group than in the no murmur group, although the general incidence of the lowered intensity was not impressive

Second heart sound Increased loudness of the pulmonary component of second heart sound, and its pathologically broad splitting were significantly ($p<0.01$) more frequent in the MIMI than the no murmur group

The increased intensity of the pulmonary component can be reasonably ascribed to the more common occurrence of left ventricular failure and to the regurgitant flow (Ross *et al* 1958, Bentivoglio *et al* 1958) The broadened splitting of second sound was always associated with accentuation of the pulmonary component, in the no murmur group its occurrence was quite infrequent

A shortened left ventricular ejection time causing earlier closure of the aortic valve in mitral incompetence (Bridgen and Leatham 1953, Perloff and Harvey 1962, Humphries 1964) would readily explain the splitting Nixon and Wagner (1962), however, presented evidence of another type of response of the left ventricle in mitral regurgitation If the end diastolic pressure was elevated the duration of the ejection of the left ventricle was at its upper normal range or prolonged The probable mechanism might

also be left ventricular failure leading to failure of the right ventricle and thus to its prolonged ejection time Both mechanisms would be consistent with the intimate relation of splitting to accentuation of the pulmonary component

The association of broad splitting with accentuation of the pulmonary component of second sound in the MIMI group would indicate that regurgitation is not wholly insignificant, as it adds to pulmonary venous hypertension

No paradoxical splitting of second heart sound (Yurchak and Gorlin 1963) was recognized Friedberg (1966) criticized reports of paradoxical splitting in infarction assuming the error to be in the misinterpretation of atrial gallop and first heart sound as paradoxical splitting

Paradoxical cardiac pulsation is an invariable result of an ischemically damaged heart in experimental studies (Tennant and Wiggers 1935 Murray 1947, Prinzmetal *et al* 1949 Gregg 1950) This dynamic aneurysm can be recorded most reliably by kinetocardiography in up to 100 % of the patients with acute myocardial infarction (Suh and Eddleman 1959 Davie *et al* 1962 Schweitzer *et al* 1965 and often by radiological methods Schwedel *et al* 1950, Sampson *et al* 1956, Kurtzman and Lofstrom 1963, Tumanovsku and Garmash 1963) However, this has been reported as occurring very infrequently (5 30 %) as a clinical palpation finding (Vakil 1955 Suh and Eddleman 1959 Davie *et al* 1962, Rorvik 1963 Logue and Hurst 1966 b)

In the present study, however, the recognition of paradoxical pulsation, often of a marked degree was frequent (51 %) and was an impressive finding revealed by careful examination A kinetocardiographic comparison of 40 patients resulted in a close correlation to the palpatory finding

Paradoxical pulsation developed significantly more often ($p<0.01$) in the MIMI group There was a close relation between the onset of paradoxical pulsation and the onset of mitral incompetence Often, even in daily examination of a patient, a paradoxical pulsation on palpation and the emergence of the murmur of mitral incompetence in ausculation in a previously murmurless heart, might appear on the same day as a new finding The cause is not so evident, for the appearance of a dynamic aneurysm from ischemic damage, the onset of mitral incompetence and heart failure were all intimately related in terms of time to each other

It is evident that either mitral incompetence (Tucker *et al* 1955, Davie *et al* 1962, Delyannis *et al* 1964, Logan *et al* 1967) or heart failure (Davie *et al* 1962, Harrison 1965) has a distinctly predisposing effect on the appearance of palpable paradoxical pulsation Probably, larger volume changes in addition to underlying ischemic dynamic aneurysm are involved

To sum up there seem to be some factors related to inefficient microcirculation of subendocardial layers of the left ventricle including papillary muscles, which have a predisposing effect on the development of mitral incompetence as a complication of acute myocardial infarction

Acute initial hemodynamic changes, or the severity of infarction, are not related to the development of MIMI This stresses the importance of the site rather than the size of the infarction, as, for example, damage to the papillary muscle, or its supporting ventricular wall, which play an important part in adequate mitral valve closure Although clinically usually slight in degree, MIMI is quite frequently associated with various clinical findings pointing to failure of the left ventricle

Mitral incompetence following acute myocardial infarction had a very wide clinical spectrum Severe mitral incompetence superimposed acutely on myocardial infarction may lead to fatal pulmonary edema On the other hand, a transient mitral systolic murmur without any associated clinically detectable disturbances might be the sole manifestation of this complication

COMPARISON OF CLINICAL FINDINGS IN PATIENTS WITH AND WITHOUT MITRAL INCOMPETENCE

ELECTROCARDIOGRAPHY

METHODS

APPARATUS AND PROCEDURE

Electrocardiograms were taken by the clinical laboratory as a hospital routine Direct writing ink jet electrocardiographs were generally used Conventional 12 leads I II III aVR aVL aVF and V_{1-6} were taken during the first four days daily and thereafter on every fifth day of each patient s stay in hospital The paper speed was 50 mm/sec The calibration was checked after every recording The synchronism of the simultaneously recorded canals could be checked from simultaneous calibration deflections The correction for inappropriate calibration was always made in the final calculation of amplitudes

ANALYSIS AND MEASUREMENTS

All the electrocardiograms were analyzed by the author for the site and extent of infarction left ventricular hypertrophy signs of left atrial overloading in the P wave and for arrhythmias and conduction disturbances

LOCALIZATION AND EXTENT OF MYOCARDIAL INFARCTION

After a review of the standard textbooks dealing with electrocardiography (Sodi Pallares and Calder 1956 Massie and Walsh 1960 Holzmann 1961 Lamb 1965) to establish the localization criteria used in myocardial infarction the diversity of methods and nomenclature soon became evident showing conflicts and inaccuracies The criteria presented by Lipman and Massie (1965) based chiefly on the investigations of Sodi Pallares and his co-workers (see Lipman and Massie 1965) seemed to be the most concise and logically stated and were therefore adopted for the purposes of this study

LOCALIZATION OF INFARCTION

ANTEROSEPTAL (middle third of the septum after Sodi Pallares) Abnormal Q wave preceding rS deflection in V_{1-3} or QS in V_{1-3} Absence of normal q in leads V_5 and V_6 The changes are ascribed to loss of activation vector I forces

ANTERIOR The presence of initial R in lead V_1 and persistent q waves in leads V_5 and V_6 (vector I forces undisturbed) The appearance of an abnormal Q wave in one or more of the next three leads V_2 V_3 and V_4 (lower third of septum) An abnormal right to-left decrease in the relative amplitudes of the R waves without their disappearance in precordial leads to the left of lead V_1 (epicardial infarction of the right lower septal mass) If an intramural portion of the right septal mass is infarcted a qR complex is recorded in leads V_3 and V_4 but the R wave appears late (0 05 second) without slurring and no peri infarction block is present QS complexes in leads V_1 to V_4 and initial R wave (absent septal q) in leads V_5 and V_6 indicate the most important infarction of the lower two-thirds of the septum (anteroseptal + anterior)

ANTEROLATERAL. Abnormal Q waves in leads V_5 and V_6 or in leads V_4 to V_6 as well as in leads aVL and I In high anterolateral localization changes are present only in leads I and aVL.

EXTENSIVE ANTERIOR (lower two-thirds of septum and infarction of left free wall) Abnormal Q waves present in all chest leads (except occasionally lead V_1) in lead aVL and in lead I

INFERIOR Abnormal Q waves in leads II III and aVF or in III and aVF In leads III and aVF rs or rSR complexes instead of Q waves

INFEROLATERAL. Criteria of inferior infarction and abnormal Q waves in lead V_6 and sometimes in leads I aVL and V_5

POSTEROLATERAL. A tall wide R wave in V_{1-2} with an R/S ratio greater than 1 and abnormal Q waves in lead V_6 and sometimes in leads I aVL and V_5

STRICTLY POSTERIOR Abnormal Q waves do not appear in any of the conventional leads RSR configuration (rSr rSR or rsR) or a tall slurred wide R wave with an R/S ratio greater than or equal to 1 in V_{1-2} Tall R waves in leads V_1 and V_2 and possibly in lead V_3 and the appearance of septal and of left ventricular morphology in leads V_{4-6}

EXTENT OF INFARCTION

The criteria of transmural and also of epicardial

nd intramural infarction have been presented in the foregoing

Subendocardial infarction does not generally produce pathological Q waves (Cook *et al* 1958) Diagnosis is based on typical ST segment and T wave changes in serial tracings In this study the extent of myocardial infarction was classified as transmural or subtransmural the latter designation including patients with electrocardiographic signs of subendocardial intramural or epicardial infarction

ABNORMAL Q WAVES

The criteria of abnormal Q waves as presented by Goldberger (1951) Barker (1952) and Sodi Pallares and Calder (1956) were used in the analysis of Q waves

LEFT VENTRICULAR HYPERTROPHY

The QRS-criteria of Manning and Smiley (1964) for left ventricular hypertrophy were adopted The heigths of the R waves in leads I and III aVL V_5 and V_6 were measured and the sums of the R waves and of the S wave in V 1 and the R wave in lead V_5 or V_6 calculated.

LEFT ATRIAL OVERLOADING

The following currently accepted methods of P wave analysis were used P wave duration P terminal vector in the frontal plane and P terminal force V_1 (Morris *et al* 1964)

The duration of the P wave in standard limb leads usually lead II was measured A duration of over 0 11 seconds was considered pathological (Lipman and Massie 1965) No correction was made for the heart rate or age of the patient

P terminal vectors in the frontal plane were calculated from the P waves in leads I and II and in leads aVL and aVF The mean of these two vectors usually of about the same magnitude was utilized as the final vector angle The amplitudes were measured to an accuracy of 0 1 mm from the top of the atrial deflection to the top of the base line if the form and timing of the peak indicated it to be definitely in the terminal part of the P wave If the P wave was rounded its height was measured at the line of division between the middle and the last third of the P wave duration which was usually 0 03 to 0 04 seconds backwards from the cessation point of the P wave deflection This method was used to obtain the amplitude of the pure terminal part of the P wave and not the summation of the initial and terminal parts of the P wave (P wave mean vector Sodi Pallares *et al* 1965) The amplitudes were measured by means of a fine lined grid and applied to a hexaxial system to estimate the magnitude of the vector angle (Grant 1957) The symmetric hexaxial system can be better used for atrial vectors (Holzmann 1961) than the corrected axial system for QRS vectors (Lamb 1965) An angle of −30 of the P terminal vector in a frontal plane was used as the upper limit of the normal (Gooch *et al* 1966)

P terminal force in the horizontal plane Precordial lead V_1 was used for measurements. The P terminal force was calculated from the duration and amplitude of the terminal deflection of the P wave as described by Morris *et al* (1964) By means of a fine lined grid the small durations and amplitudes were quite easily and reproducibly measured

The terminal P wave vectors in the frontal plane and P terminal forces were analyzed from tracings recorded before MIMI on some of the first 5 days usually days 1–3 from the onset of infarction Values after the development of MIMI were obtained from tracings taken during the 3–4 weeks subsequent to the onset of infarction In patients without any murmur the tracings of a comparable time were used In patients who died in hospital it was usually necessary to utilize the second tracing taken earlier than the third or fourth weeks after the onset of infarction or at least one taken following the development of mitral incompetence The third calculation was made from the follow up electrocardiograms.

Values of over − 0 03 mm sec were considered pathological

COMMENTS

Myocardial infarction The reliability in the study of Simonson *et al* (1966) was as follows The presence of infarction was recognized least frequently (60 %) in posterior wall infarction and most frequently in anterior+posterior wall infarction (94 %) while in anterior wall infarction a correct diagnosis was made in 68 % Of the total material 84 % of the myocardial infarctions were recognized solely on the basis of an electrocardiogram False positives in the frequency of 13 % were caused by a bundle branch block and by left ventricular hypertrophy Thirteen studies reviewed showed a reliability varying between 22 and 100 % according to post mortem examinations

The accuracy of right localization of myocardial infarction was much lower than recognition of infarction from one electrocardiographic tracing being about 50 % regardless of site (anterior posterior anterior+posterior and subtotal were analyzed) Several authors state that the reliability of electrocardiographic localization varies considerably with the site of infarction being from 100 % in lower septal to 0–5 % in lateral infarctions (Sodi Pallares *et al* 1963 Pruitt *et al* 1963)

The diagnostic accuracy of electrocardiography in myocardial infarction is limited by the size and site of the infarction several simultaneous infarctions (causing the phenomenon of electrical countrecoup) conduction disturbances, hypertrophy of the left ventricle and previous ischemic changes (Pruitt *et al* 1963)

On the other hand the electrical changes in serial tracings typical of ischemic injury and necrosis and restitution considerably facilitate electrocardiographic diagnosis of infarction at the acute stage

The foregoing comments refer to the diagnostic accuracy of electrocardiography It is evident that supported by the clinical picture and the laboratory results the diagnosis of acute myocardial infarction will substantially improve This does not of course apply to the localization of infarction for which electrocardiography remains the only clinical procedure

Left ventricular hypertrophy The QRS criteria of Manning and Smiley (1964) were considered to give the most reliable results because they take into account the physique which can substantially alter the amplitudes especially of precordial tracings (Kilty and Lepeschkin 1965)

In comparing study of the accuracy of electrocardiography with vectorcardiography made by Simonson *et al* (1966) a correct diagnosis of left ventricular hypertrophy was made in 67 % of the cases by ECG In 15 articles reviewed in this paper the reliability of the electrocardiographic diagnosis of left ventricular hypertrophy on the basis of autopsy material varied in larger series between 32 and 76 % Most of the authors used the criteria of Sokolow and Lyon (1949) The false positivies accounted for 3 9 % in the study of Simonson *et al* (1966) in the papers reviewed this was 0—8 % by the QRS criteria only

In the present study of myocardial infarction the sensitivity of the electrocardiogram in detecting left ventricular hypertrophy is obviously diminished to a marked degree ST segment and T wave changes cannot be used and the sensitivity of QRS criteria is diminished by the loss of viable muscle mass

Left atrial overloading Many calculations have been proposed for the establishing of reliable criteria for left atrial enlargement (see Morris *et al* 1964) Of these the P terminal force described by Morris *et al* (1964) is simply accurately and reproducibly measured (±0 01 mm sec) and its sensitivity as an indicator of left atrial over loading seems to be quite good (Gooch *et al* 1966) The P terminal force mainly measures the electrical force of the left atrium in a horizontal plane Any cardiac electrical vector has a spatial direction In addition an atrial dilatation may take different directions To obtain a comparison of vector magnitudes and their alterations also in the frontal plane they were calculated according to the hexaxial system The method is however rather cumbersome and possesses a limit of accuracy of only about 15 (Hiss *et al* 1960)

RESULTS

ELECTROCARDIOGRAPHIC SIGNS OF MYOCARDIAL INFARCTION

Localization (Table 15, Fig 26) In 117 patients developing mitral incompetence as a complication of myocardial infarction (group 1), there was no difference in the incidence of anterior (anteroseptal anteroseptal + anterior, anterior, anterolateral, extensive anterior) localization of infarction (51 %) as compared to posterior (inferior inferolateral inferoposterior, inferoposterolateral, posterolateral, strictly posterior) localization (49 %) On the other hand in the no-murmur patients (group 2) a marked difference did exist, consisting mainly of anterior infarctions (73 %), posterior localization in this group being much less frequent (27 %), as judged by electrocardiography

In the MIMI group the relative frequencies of all the sublocations of posterior infarction and extensive anterior infarction were greater than in the no murmur group, where all the other anterior sublocations were more common

Extent (Table 15) Transmural myocardial infarction occurred in a total of 144 of 187 patients (77 %), and the total frequencies were equal in both MIMI and no murmur groups Transmural infarction was slightly more common (56 %) in the posterior location in MIMI patients, and significantly more common (72 %) in the anterior location of no-murmur patients The anterior location was much more commonly recognized by electrocardiography in subtransmural infarctions involving both groups of patients

LEFT VENTRICULAR HYPERTROPHY

A diagnosis naturally had to be based solely on QRS criteria, and most often only precordial leads met them The ECGs of 33 patients (18 %) met the criteria of Manning and Smiley (1964) for left ventricular hypertrophy and of these all but 5 further met the much

Table 15 *Location and extent of myocardial infarction according to electrocardiography*

Location of infarction	Mitral incompetence			No murmur			Total
		Trans mural	Subtrans mural		Trans mural	Subtrans mural	
Anteroseptal	1 (1 %)	0	1	3 (4 %)	2	1	4 (2 %)
Anterior	8 (7 %)	6	2	6 (9 %)	5	1	14 (7 %)
Anteroseptal – anterior	20 (17 %)	13	7	17 (24 %)	13	4	37 (20 %)
Anterolateral	11 (9 %)	4	7	12 (17 %)	7	5	23 (12 %)
Extensive anterior	20 (17 %)	17	3	13 (11 %)	12	1	33 (18 %)
Total anterior	60 (51 %)	40	20	51 (73 %)	39	12	111 (59 %)
Inferior	17 (15 %)	15	2	5 (7 %)	4	1	22 (12 %)
Inferolateral	16 (14 %)	13	3	6 (9 %)	3	3	22 (12 %)
Inferoposterior	6 (5 %)	5	1	2 (3 %)	[illegible]	0	8 (4 %)
Inferoposterolateral	12 (10 %)	12	0	5 (7 %)	5	0	17 (9 %)
Posterolateral	5 (4 %)	4	1	0 (0 %)	0	[illegible]	5 (3 %)
Strictly posterior	1 (1 %)	1	0	1 (1 %)	1	0	[illegible] (1 %)
Total posterior	57 (49 %)	50	7	19 (27 %)	15	4	76 (41 %)
Total	117	90	27	70	54	16	187

used criteria of Sokolow and Lyon (1949) In the MIMI group there were 22 patients (19 %) and in the no-murmur group 11 patients (16 %) in whom an electrocardiographic diagnosis of left ventricular hypertrophy could be made during the acute phase of infarction



Fig 26 *Relation of the site of the myocardial infarction as judged by electrocardiography to the presence or absence of mitral incompetence*

LEFT ATRIAL OVERLOADING (Table 16 Figs 27–31)

The duration of the P wave was over 0 11 sec in a total of 68 patients (36 %) This was observed in 39 % of the patients in the MIMI group and in 31 % of the ones without any mitral systolic murmur The duration was longest mostly in lead II The form of these P waves of long duration was mainly bifid or double peaked but only rarely were they of high amplitude

P terminal vector in the frontal plane (Table 16, Fig 27) Calculations were made from the data on 99 patients with acute mitral incompetence where electrocardiograms were available before and after the development of MIMI Analyses of 63 no murmur patients were made in a comparable time

On the average the vector angle in the MIMI group did not differ significantly from that in the no murmur group before discovery of the mitral systolic murmur and after the appearance of the mitral incompetence, whereas at follow up examination it was significantly lower

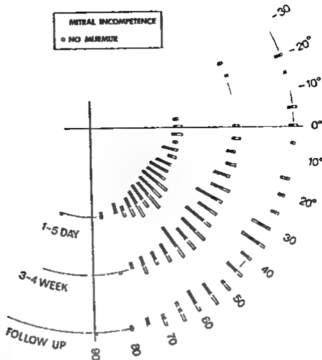
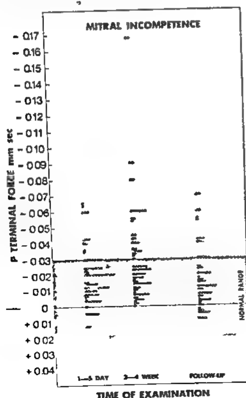


Fig 27 *Terminal P wave vectors in the frontal plane related to mitral incompetence following acute myocardial infarction No apparent change was present*



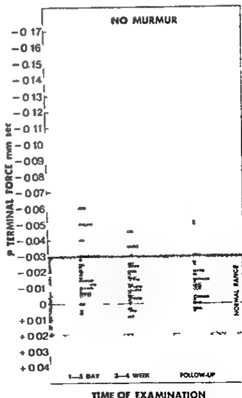


Table 16 *P wave changes of left atrial overloading in patients with and without mitral incompetence*

Time of examination	Mitral incompetence	No murmur	P
	P terminal vector in frontal plane degrees		
1–5 day	41 ± 21	45 ± 27	N S
3–4 week	40 ± 20	39 ± 23	N S
Follow up	38 ± 25	46 ± 24	<0.05
	P terminal force mm sec		
1–5 day	*0.021 ± 0.029*	*−0.021 ± 0.019*	*N S*
3–4 week	0.031 ± 0.032	−0.017 ± 0.021	<0.001
Follow up	0.022 ± 0.026	−0.014 ± 0.018	<0.05

N S non significant

in the MIMI group than in the no *murmur group*

There was no notable shift between the different *periods in* either group The inci dence of pathological P terminal frontal vectors less than +30 before and after the development of MIMI and on follow up was as follows before MIMI *on some* of days 1–5 22 %, after MIMI in the third or fourth weeks, 21 % on follow up 25 % and no murmur 22 %, 26 % and 21 % respectively

P terminal force (Table 16, Figs 28—31) In contrast to the rigid picture presented by the vectors in the frontal plane, marked changes were observed in the P terminal force in studying the values before and after the appearance of the *mitral incompetence*

In statistical analysis, the difference be tween the MIMI and no murmur groups was *non significant in the initial* phase, but highly *significant* (p 0.001) *during the* third or fourth week and propably signi ficant (p < 0.05) on follow up In the MIMI group, the mean P terminal force was significantly (p 0.01) higher after the development of *mitral incompetence* than before its onset At corresponding times of examination no significant difference existed in the no murmur group The diffe rence was also clearly present in the study of the incidence of pathological values over −0.03 mm sec Morris *et al* 1964) Fig 29 The initial situation was the same in *the* MIMI *(21 %) and in the no murmur* group (23 %), but after the appearance of the mitral incompetence a substantial *increase* was observed *in the* MIMI group (39 %) while in the no murmur group during the corresponding time the patho logical values *declined* (17 %) The dif ference remained during the follow up phase, in the MIMI group there was a slight decline (33 %) and in the no-

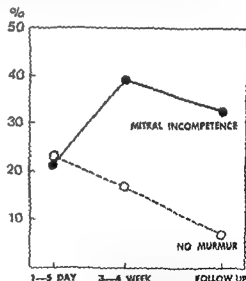


Fig 29 *Incidence of the pathological P terminal forces (over 0.03 mm sec) in patients with or without mitral incompetence*

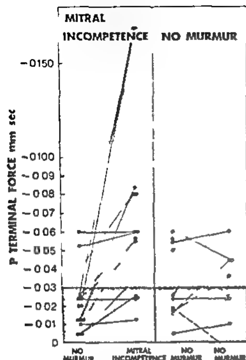


Fig 30 *Values of P terminal forces of the patients dying in hospital related to the presence or absence of mitral incompetence*

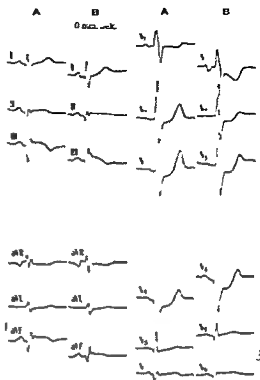


Fig 31 *Electrocardiograms recorded before (A) and two days following (B) the onset of an acute mitral incompetence as a sequela of an infero posterolateral myocardial infarction Note the acute development of a markedly pathological P wave in lead V_1. The P terminal force changed from −0 025 to −0 17 mm sec The P terminal vector in the frontal plane was not conspicuously changed*

murmur group a steeper one (7 %)

In statistical analysis where the presence or absence of mitral incompetence was related to P terminal force in the pooled MIMI and no-murmur groups the groups differed at the significance level of $p < 0.05$ in the third or fourth weeks and $p < 0.001$ on follow up

The same trend in the increase of P terminal force is seen in Fig 30 which refers to the patients of the MIMI and no-murmur groups who died in hospital In the MIMI group a distinct acute increase in the normal or pathological initial value occurred, the increase from a mean of −0 025 to −0 071 mm sec being statistically significant ($p < 0.01$) In the no-murmur group the change was not consistent, in many instances, no change could be seen or even a decline took place and in the statistical calculation the change from a mean of −0 032 to −0 042 mm sec was non significant

There is reason to emphasize the occurrence of strong shifts even during a few days observation (Figs 30, 31)

In addition to the extra burden imposed on the left atrium by acute mitral incompetence, changes of blood pressure values (Sodi Pallares and Calder 1956, Lubeck *et al* 1963, Ross 1963) and heart failure (Sutnick and Soloff 1962, Mori and Mazzei 1965, Sitar 1965) could also produce similar effects In determining the part played by heart failure in addi

tion to mitral incompetence, the changes in the P terminal force determined by bi serial correlation coefficients of P terminal force/mitral incompetence and P terminal force/congestive heart failure were about the same, −0 24 and −0 25 (n 163) in the third or fourth weeks of illness, and −0 27 and −0 31 (n 126) on follow up The systolic blood pressures did not differ significantly in either patient groups when the values on some of days 1−5 from onset were compared to pressures measured during the third and fourth weeks after onset of infarction It may be worth repeating that the values of the P terminal force changed inconsistently in patients who died in the no murmur group although they had in many instances had very marked heart failure (Fig 30)

OTHER ELECTROCARDIOGRAPHIC FINDINGS

Total LBBB occurred in the MIMI group 3 times, and in the no murmur group once while the total RBBB occurred twice in either group Right atrial overloading was observed once in both groups Simultaneous total LBBB and RBBB was not found An intraventricular conduction disturbance with a QRS duration of 0 12 sec or more, which could not be classified as a bundle branch block on either side was noted in 5 MIMI and 3 no murmur patients Peri infarction blocks were not analyzed Intraventricular conduction disturbances seemed to be a bad omen, all but 1 of 8 patients who had or developed this electrocardiographic sign died in hospital

DISCUSSION

The location of infarction Contrary to the earlier statements that posterior myocardial infarction is a much commoner cause of mitral incompetence (Nezlin and Shamesova 1951 Froment *et al* 1955, Bashour 1965) or that the anterior location wholly predominates (Burch *et al* 1963, Phillips *et al* 1963 a, b, Segal and Likoff 1964), no difference in the frequencies of anterior and posterior site could be found in the present investigation as MIMI patients were concerned

This discrepancy might be explained by the more common occurrence of transient, faint and atypical murmurs of mitral incompetence in anterior infarction (see Chapter VI) Such murmurs may have been missed without daily auscultation, or explained on other grounds In posterior infarction the murmur tended to be more typically pansystolic at the apex as well as louder and more constant This is consistent with the more common transmural extent of posterior infarction, leading perhaps to more severe papillary muscle damage (Chapter IX) than when the anterior wall was involved Otherwise the anterior location was significantly more common in the no murmur group

These findings nevertheless finally indicate that posterior infarction is a commoner cause of MIMI than anterior infarction, although the difference is clear only in the total patient series and in the degree of mitral incompetence

Hence the results of the present study partially combine the findings of reports ascribing predominantly posterior or predominantly anterior location of the lesion in mitral incompetence to the involvement of the papillary muscle (Chapter II) Posterior infarction seems to destroy the neighbouring papillary muscle more extensively than does anterior infarction Less severe mechanical dysfunction of the ischemic anterior papillary muscle often causes only transient and slight regurgitation Vulnerability and more extensive destruction of the posterior than of the anterior papillary muscle was definitely evident in the present autopsy study (Chapter IX)

Left ventricular hypertrophy There was no difference in the frequency of the left venticular hypertrophy between the MI-

MI group and the no murmur patients during the acute phase of infarction However, a marked tendency to develop or exaggerate previous signs of LVH in the MIMI group was observed in the follow-up phase (Chapter X)

The low frequency of positive findings as compared to the incidence of hyper tension and to the autopsy findings stresses the unreliability of electrocardiographic diagnosis of this condition in the presence of myocardial infarction

Left atrial overloading The P wave is generally assumed to be generated by electrical activation of the right and left atrial musculature (Hecht and Woodbury 1950, Wenger and Hofmann Credner 1952, Reynolds 1953, Puech *et al* 1954 Sodi Pallares *et al* 1965), although a radically controversial explanation has recently been presented (Radner 1966)

Activation of the left atrium occurs normally 0 04—0 05 seconds after the onset of the right atrial activation Thus pathological changes in the terminal por tion of the P wave have been considered to reflect the verified changes of left atrial hypertrophy, dilatation or muscular damage Changes have been most often observed in acquired valvular diseases the most extreme changes occurring in mitral stenosis A good correlation has been usually found in pathological, radiological and hemodynamic studies between the structural and functional alterations un dergone by the left atrium and the changes occurring in the P wave (Berliner and Master 1938 Hecht and Woodbury 1950, Bradley and Marriot 1956, Soloff and zatuchni 1958 Dines and Parkin 1959 Arwidsson *et al* 1960, Morris *et al* 1964)

Evidence of rapid and sensitive P ter minal wave changes has recently emerged in hypertension, left ventricular failure myocardial infarction and exercise These have been explained on the grounds of left atrial strain or dilatation and a posterior shift of the terminal P wave vector (Sutnick and Soloff 1962 Lubeck *et al* 1963 Ross 1963, Cortes 1963, Sitar 1965, Desrochers and Proulx 1965)

Only a few reports have been published on P wave alterations in acute myocardial infarction (Master 1933, Bloom and Gilbert 1942 Vill 1949 Katz 1949, Lepeschkin 1957, Gross 1963, Miori and Mazzei 1965, Sitar 1965) Increased duration or notching of the P wave is said to occur infrequently and is attributed to acute left ventricular failure or increased sympathetic tone

However, in the only two systematic investigations carried out in addition to Master s (1933) Miori and Mazzei (1965) and Sitar (1965) observed the P wave alterations to be quite common in acute myocardial infarction The P wave re corded in lead V_1 appeared to reflect the pathological changes most sensitively These changes were ascribed to failure of the left ventricle In many instances, they were transient To the more perma nent changes Sitar (1965) applied a guarded prognosis, as had previously been done by Bloom and Gilbert (1942)

The results of the present investigation point to frequent and very rapid changes in the P terminal force in acute myocardial infarction This sign of probable left atrial strain developed significantly more frequently and was more marked in degree among MIMI patients than those of the no-murmur group

The P wave on admission was often pathological also in the no-murmur group Later normalization of the P terminal force in this group would suggest in the main only transient and reversible initial hemodynamic strain caused by the acute myocardial damage The same relationship was noted in the atrial gallop (Chapter VII A)

By contrast in the MIMI group the electrocardiographic changes in the P wave progressed after the development of mitral incompetence This is reasonable when one considers the added volume of work of both the left atrium and the

left ventricle with the frequent develop ment of congestive heart failure leading to a rise in both the volume and the pressure loads in the left atrium

The P terminal force was superior in sensitivity when compared to the change of direction of the P terminal vector in the frontal plane The findings of Abilskov 1957) and of Sutnick and Soloff (1962) pointed in the same direction in compar ing the P terminal force to the mean atrial vector When the P terminal force took a pathological course the P terminal vector in the frontal plane shifted — not always toward lower but often also toward higher values, and vice versa This could be explained by the major change being a posterior rotation of the P vector in the horizontal plane (Sano *et al* 1957 Martins de Oliviera and Zimmer man 1959, Sutnick and Soloff 1962 Morris *et al* 1964) whereas a simultaneous change in the frontal plane might have been up or down

The results of these very acute terminal P wave changes in the present study are contrary to the observations of Gooch *et al* 1966) who found the frontal plane terminal vector to be more sensitive than the P terminal force Their patients how ever had a conspicuously enlarged left atrium as a consequence of valvular heart disease Altered anatomical size and position of the left atrium might affect the P wave differently from the acute electromechanical strain in a pre-dominantly normal sized left atrium An insignificant shift of the mean P terminal vector in the frontal plane to the left was observed in the MIMI group This shift was in the opposite direction to the deviation of the mean P wave mean vector in coronary patients observed by Gross (1963) to be to the right from normal This change was ascribed to chronic ischemic damage and thus to loss of electrical force generated by the left atrium

The acute marked changes of the P terminal force in a pathological direction, as observed in the present study, had the same significance pointing to a guarded prognosis as in the findings of Sitar (1965)

To sum up the frequency of electrocardio graphic location of the anterior and posterior myocardial infarction was equal in patients who developed mitral incom petence after acute myocardial infarction The anterior infarction dominated in the group without mitral incompetence Sub-endocardial infarction can well be asso-ciated with development of mitral incom petence The magnitude of P terminal force was significantly correlated with development of MIMI and often rapid changes seemed to have some prognostic significance

COMPARISON OF CLINICAL FINDINGS IN PATIENTS WITH AND WITHOUT MITRAL INCOMPOTENCE

RADIOLOGY

METHODS

All the patients underwent a radiological examination of the chest during the mobilization stage of treatment 2 to 4 weeks after the onset of infarction The radiographs were studied to estimate the volume of the heart the degree of enlargement of its chambers the changes in the large blood vessels and particularly the pulmonary signs of left sided heart failure

The generally used teleradiographic ellipsoid approximation method of measuring the *volume of the heart* (Liljestrand *et al* 1939 Jonsell 1939) was applied in the present study with certain modifications The pictures were taken according to routine clinical procedure in the erect position with moderate inspiration and without correlation to the cardiac cycle The exposures for postero anterior and lateral pictures were not simultaneous The focal distance was chiefly 1 5 meters for both projections but in one clinic some of the patients were examined with a 2 0-m focal distance Therefore to obtain comparable volumes a correction calculation for magnification caused by diver gent rays was made (Klepzig and Frisch 1965) The final coefficients used including magnification and assumed ellipsoidal shape of the heart were 0 41 for the 1 5 m and 0 44 for the 2 0 m focal distance Domenet *et al* (1963) and Evans and Carpenter (1963) used the constant 0 41 after studying a large number of subjects to ascertain among other things the influence of the heart film distance Jonsell (1939) had a slightly higher value of 0 42 and Amundsen (1959) a slightly lower one of 0 40

In order to achieve maximum accuracy in calculating the heart volume the present author outlined the the heart as a figure resembling an ellipse (Klepzig and Frisch 1965) On the top of this drawing a regular ellipse drawn on a transparent sheet and measuring 12 × 16 cm was placed By means of this model which was provided with centigrade longitudinal and transverse axes at right angles it was possible to place rapidly and accurately on the heart shadow itself the longitudinal and transverse axes situated perpendicular to each other The depth was measured from the anterior border of the heart in the direction of the beams to the posterior border or if this was not visible to the border indicated by the barium swallow

Heart volumes were calculated in relation to the body surface area This was obtained from a nomogram based on the formula of DuBois and DuBois (1916)

The size of the *chambers of the heart* was graded from 0 to 3 as follow

Left atrium
- 0 — normal
- 1 — slight but no longer normal identation in the lateral view of the esophagus
- 2 — distinct displacement of the esophagus with the auricular appendage often visible
- 3 — prominent enlargement of the atrium and a double contour at the border of the right auricle

Left ventricle
- 0 — normal
- 1 — slight rounding of the apex and a straightening-out of the ventricle-diaphragm angle
- 2 — distinct enlargement and displacement downward or outward in frontal and lateral views as well as increased heart volume
- 3 — marked enlargement

Right atrium and ventricle

Corresponding principles were applied as in estimating the size of the left atrium and ventricle

The signs of *left sided heart failure in the pulmonary circulation* were estimated according to the principles presented by Short (1955) Grainger (1958) Steiner (1958 1959) Harley (1961) Simon (1961) Lavender and Doppman (1962) Lavender *et al* (1962) Logue *et al* (1963) as follows
- 0 — normal
- 1 a — venodilatation in the lower and middle fields and/or slight hilar or pulmonary clouding
- 1 b — marked venodilatation in all the pulmonary fields and/or distinct clouding
- 2 — venoconstriction in the lower fields attended by marked venodilatation in the upper fields causing reversal of the normal vascular pattern Septal or A lines and/or hydrothorax

3 — distinct alveolar pulmonary edema

The diameter of the right descending branch of the pulmonary artery was measured as far as its limits could be distinguished in the middle of the interstitial edema

All the estimations were made as double determinations expecting those involving heart volumes in which the double determinations were made to measure the intra-observer error from 52 pairs of pictures selected at random after earlier markings had been wiped off No inter-observer error was determined as the analyses were made by the author alone

Relative heart volumes of over 500 ml/m² of BSA for men and over 450 ml/m² of BSA for women were considered pathological (Liljestrand *et al* 1939 Jonsell 1939 Maurea *et al* 1955 Nylin 1957 Musshoff *et al* 1958 Amundsen 1959)

ACCURACY OF RADIOLOGICAL HEART VOLUME MENSURATION

Technical considerations

The ellipsoid approximation method of heart volume determination seems to be astonishingly accurate when tested by comparing the radiological volume with the autopsy displacement volume or with tomographical calculations (Lind 1950 Friedman 1951 Gebhardt 1957 Evans and Carpenter 1965) the error being under 5 %

The reproducibility from double determinations is good showing deviations of 3–5 % (Axén *et al* 1946 Kjellberg *et al* 1951 Linderholm and Strandell 1958 Amundsen 1959 Holmgren and Ovenfors 1960) In the present study the significant (at a level of $p < 0.001$) discriminant volume was 20 ml as determined from duplicate countour drawings and calculations from 52 unselected pairs The mean absolute heart volume was 947 ml in the first and 962 ml in the second estimation The difference ranged from 0 to 90 ml and the standard error of the change was 6 1 ml For relative volumes the error is obviously considerably smaller

Adherence to fixed points in measuring the long and the short axes of the heart as described by Jonsell (1939) has obvious disadvantages if no correction is made for the axis deviation from the right angle The method used here for perpendicular axis determinantion on a drawn ellipse in comparison with the routine radiological procedure of measuring them from fixed points without any angle correction often yielded a difference of as much as 100 ml in heart volume

Physiological factors

The error originating from the heart cycle in the determination of the heart volume as revealed by a comparison of pictures taken during peak systole and diastole is reduced when unsynchronized pictures are taken at random Similarly normal respiration has no appreciable effect on volume (Jonsell 1939 Lind 1950 Kjellberg *et al* 1951 Nylin 1957)

The position of the subject under examination often has considerable effect on the volume in an upright position the heart is smaller and more sensitive to any change in the heart volume depending upon orthostatic changes in venous return and heart rate (Kjellberg *et al* 1949 Kjellberg 1952 Musshoff and Reindell 1956 Linderholm and Strandell 1958 Holmgren and Ovenfors 1960) Physical training markedly affects the heart volume which increases with an increase of red blood cell mass (Kjellberg *et al* 1949 Musshoff *et al* 1958)

Axén *et al* (1946) investigated the total error of the method including its technical and physiological aspects and obtained a result of only 3 4 %

RESULTS

Heart volume The pictures were taken 2—4 weeks after the onset of infarction 101 (86 %) pictures involving MIMI and 61 (87 %) involving no-murmur patients were available, for patients who died in hospital before the mobilization stage, no pictures were usually available

The heart size was about of the same magnitude in both MIMI and no murmur groups (Tables 17 and 18) The heart volumes were over 500 ml/m of BSA in 68 of 162 patients (42 %), 45 of them (45 %) from the MIMI group and 23 (38 %) from the no-murmur group The enlargement was usually moderate 600 ml/m of BSA being exceeded by only 12 MIMI and 8 no murmur patients In the majority (55 %) of the MIMI patients the heart volume was notably within normal limits about 2 or 3 weeks after the murmur had developed (Figs 32 and 33)

The differences in heart volume in patient groups with anterior myocardial infarction were immaterial (Tables 17 and 18) but with posterior infarction the heart size in the MIMI group was significantly larger than in the no murmur group In the MIMI group, enlarged hearts were found in 39 % with anterior infarction and in 51 % with posterior infarction

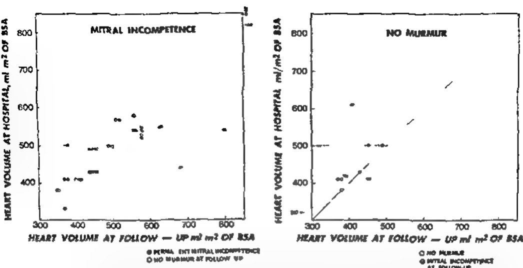


Fig 32 *Heart volumes in hospital and on follow-up patients with and without mitral incompetence 500 ml/m² of BSA indicated by the dotted lines was used as an upper limit of the normal Over half the MIMI patients had a normal heart volume in hospital with a slight tendency to enlarge on follow-up*

Table 17 *Heart volumes in patients with and without mitral incompetence*

	Heart volume ml/m² of BSA mean ± S D					
	Anterior infarction		Posterior infarction		Total	
	At hospital	At follow up	At hospital	At follow up	At hospital	At follow up
Mitral incompetence	487 ± 125 n 56	510 ± 149 n 49	528 ± 101 n 45	521 ± 91 n 37	505 ± 308 n 101	515 ± 126 n 86
No murmur	517 ± 124 n 42	501 ± 116 n 35	454 ± [illegible] n 18	460 ± 79 n 17	498 ± 113 n 60	488 ± 106 n 52
P	N S	N S	< 0 001	< 0 05	N S	N S

N S non-significant



Fig 33 *Gradual enlargement of the heart in a patient with mitral incompetence as a sequela of acute myocardial infarction At the onset of the murmur heart volume was normal At autopsy despite marked enlargement of the heart circumference of the mitral annulus was normal (95 mm) Note also the appearance of signs of progressive pulmonary venous congestion*

Table 18 *Size of heart related to site of infarction in patients with and without mitral incompetence*

Heart volume ml/m² of BSA	Mitral incompetence		No murmur	
	Location of infarction		Location of infarction	
	Anterior	Posterior	Anterior	Posterior
[illegible]	34 (61 %)	22 (49 %)	24 (56 %)	14 (78 %)
[illegible]	22 (39 %)	23 (51 %)	19 (44 %)	4 (22 %)
Total	56	45	43	18

Table 19 *Relationship between radiological pulmonary signs of left sided heart failure and mean pulmonary capillary wedge pressures obtained at cardiac catheterization*

Radiological finding	Mean pulmonary capillary wedge pressure mmHg 0–10	11–20	21 or over	Total
Venous dilatation				
slight	5 (56 %)	7 (58 %)	1 (25 %)	13
marked	2 (22 %)	4 (33 %)	3 (75 %)	9
Venoconstriction	0	2 (17 %)	2 (50 %)	4
Septal lines	2 (22 %)	5 (42 %)	4 (100 %)	11
Hilus clouding	4 (44 %)	10 (83 %)	4 (100 %)	18
Pulmonary clouding	4 (44 %)	5 (42 %)	4 (100 %)	13
Total	9	12	4	25

The radiological heart volume of patients who died in hospital was usually large Of the 21 MIMI patients who died 7 underwent a radiological examination 5 of them being found to have an enlarged heart while 5 of the 11 fatal cases in the no murmur group were radiologically examined 3 of them being found to have a heart volume over 500 ml/m of BSA

Size of the left ventricle (Fig 34) No differences were observed between the groups of patients, the enlargement generally being only slight A distinct grade 2–3 enlargement was registered in 45 % of the patients in the MIMI group and in 34 of those in the no murmur group

Size of the left atrium (Fig 34) The radiological size of the left atrium, as estimated from plain chest radiographs was in the main remarkably normal in both groups of patients — 48 % in the MIMI and 53 % in the no murmur group Only negligible enlargement, with a small rounding and an identation in the esophagus, was found in an additional 20 % of the MIMI and 24 % of the no murmur patients Thus the left atrium of about three fourths of the patients in both groups was normal or nearly normal

Right atrium and ventricle The right atrium was estimated to be enlarged in 18 % of the MIMI and in 16 % of the no murmur patients, while an enlargement of the right ventricle was noted in 24 % and 21 %, respectively

Pulmonary signs of left sided heart failure (Figs 33–35) Changes in the pulmonary vessels and tissue spaces were very common among patients with acute myocardial infarction In only 30 (30 %) of

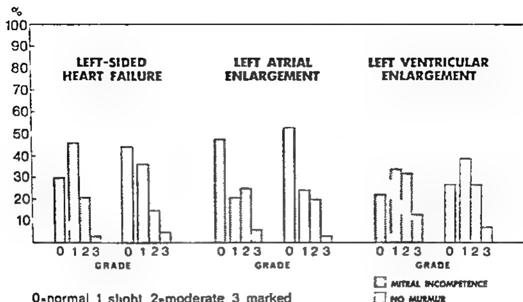


Fig 34 *Radiological findings*

the MIMI and 27 (14 %) of the no-murmur patients were definite changes in pulmonary circulation consistent with left sided heart failure estimated to be absent These figures include borderline changes and if these changes are considered to indicate incipient pulmonary venous congestion then only 7 MIMI and 6 no-murmur patients were left definitely without any radiological pulmonary changes of heart failure

Clear-cut pulmonary venous dilatation as a manifestation of pulmonary venous hypertension was observed in 56 % of the MIMI and in 45 % of the no-murmur patients Marked pulmonary venous dilatation alone in both upper and lower fields without more severe changes was

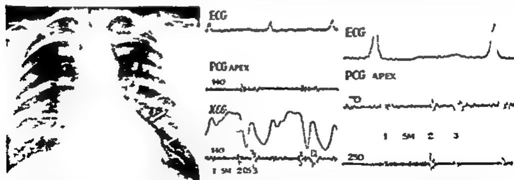


Fig 35 *A chest radiograph showing a small ventricular aneurysm confirmed by a cine fluorographic examination Severe pulmonary venous congestion A marked paradoxical cardiac pulsation was felt at the time of the onset of only grade 1 pansystolic crescendo murmur (SM) of mitral incompetence Third heart sound was easily palpable as a sudden shock also evident in the kinetocardiography Stippled area represents the pansystolic bulge*

found in 9 patients in the MIMI and 5 in the no-murmur group, these are therefore included in the number referring to slight or moderate venous dilatation grade 1)

More marked grade 2 changes in the pulmonary signs (Fig 35) were also present slightly more commonly in the MIMI 21 %) than in the no murmur group 15 %) already at this stage with the situation tending to deteriorate on follow up examination (Chapter X) Septal lines were detected in 18 % of the MIMI and 10 % of the no-murmur patients

Severe alveolar pulmonary edema was present at the time of the radiological examination in 3 MIMI and 3 no murmur patients

Most of the patients who died in hospital had evidenced conspicuous changes of left sided heart failure corresponding to grade 2—3 this was true of 9 of the 12 MIMI and 4 of the 6 no-murmur patients

The right descending branch of the pulmonary artery had a diameter of the same order in both groups, it ranged between 12—19 mm with a mean of 14 7 mm, among the members of the MIMI group and between 12—22 mm, with a mean of 14 7 mm, among those of the no-murmur group A pathological enlargement over 16 mm (Lavender and Doppman 1962) was noted in 13 % of the MIMI patients and in 8 % of the no-murmur patients

Aortic sclerosis, as estimated from plain pictures was of the same incidence in both groups No changes were found in 26 % of the MIMI or in 18 % of the no-murmur group Demonstrable aortic dilatation or visible calcification was detected in 40 % of the MIMI and in 58 % of the no-murmur patients the rest having an increased density of the degree generally interpreted as a radiological sign of aortic sclerosis

Left ventricular aneurysm of small or moderate size (Fig 35) was found in 9 % of patients in the MIMI group and in 5 % in the no murmur patients

DISCUSSION

Three important findings resulted from the radiological examination of the heart and lungs performed 2—4 weeks after the onset of myocardial infarction

Firstly, in most patients mitral incompetence developed after myocardial infarction with *the heart of normal size* Thus, the mechanism of mitral incompetence obviously could not, as has generally been assumed, be secondary to mitral annular dilatation brought on by enlargement of the left ventricle Furthermore, many of the patients in the no-murmur group were found to have considerably enlarged hearts without any sign of mitral incompetence The annular dilatation mechanism of mitral incompetence has been critized by Levy and Edwards (1962), who ascribed incompetence instead to an inappropriate alignment of the papillary muscles to a more obtuse angle in the dilating left ventricle This would necessitate a marked degree of acute enlargement Moreover, the mitral annular ring is part of the strong fibrous skeleton of the heart, where dilatation as a result of muscular damage would not be excepted

Acute left ventricular dilatation (Sarnoff and Berglund 1954, Mullins *et al* 1966) could, however, be one mechanism of regurgitation, inappropriate positioning of the papillary muscles might actually make them too short by having their attachment areas pulled down to a greater distance from the valvular orifice in the dilated ventricle (Bailas 1965) Clinical investigations of this problem are not, however, avalaible

Thus, the normal size of the heart in the majority of the patients suggests that the regurgitation mechanism represents functional or anatomical ischemic destruc-

tion of the supporting structures of the mitral valve, i e papillary muscles as most important structures

Reports on the size of the heart in cases of myocardial infarction indicate, in agreement with the findings of the present study that this can very often be normal (Waris *et al* 1966) Mitral incompetence of short duration did not apparently change the heart volume during hospitalization, the effect at follow up being a slight tendency to enlargement The finding of Froment *et al* (1955) that mitral incompetence as a sequela of myocardial infarction occurs in the presence of anterior infarction usually only in enlarged hearts (Table 1) could not be substantiated (Tables 17 and 18), the result was the opposite in the present study

Secondly, the *size of the left atrium* was mostly normal in patients who developed mitral incompetence This was also the finding in the series of acute subvalvar mitral incompetence investigated by Raftery *et al* (1966), and Kennedy *et al* (1966), as confirmed by cine cardioangiography Accordingly the normal size of the left atrium does not exclude the possible presence of even a marked degree of mitral incompetence

Enlargement of the left atrium and of the left ventricle is usually interpreted as a common and reliable radiological sign of significant mitral incompetence (Abelmann *et al* 1953 Wood 1954, Priest *et al* 1962) Nevertheless serious criticism has been directed against the exclusion of a substantial mitral incompetence on the basis of normal size of the left atrium (Bentivoglio *et al* 1961 Khalaf *et al* 1962 Braunwald and Awe 1963) Similarly, the size of the left ventricle can just as easily be normal as enlarged in patients with significant mitral incompetence (Abelmann *et al* 1953, Ross *et al* 1958 Steiner *et al* 1963) Definite enlargement of the left atrium and left ventricle is a natural consequence of any marked hemodynamic disorder straining the two chambers, but seems to be the result of a prolonged hemodynamic burden In addition, rheumatic changes in the myocardium of the left atrium and ventricle could, for example, be a factor of no little significance in causing the enlargement Consequently, any estimation of the hemodynamics of the mitral incompetence in the light of the size of these chambers is unreliable (Cobbs *et al* 1957, Shillingford 1962, Logan *et al* 1967), and, as far as acute changes are concerned quite impossible

Thirdly subtle *radiological signs of left-sided heart failure* after a myocardial infarction were extremely frequently present, and in more severe form in patients with acute mitral incompetence, further worsening in MIMI patients during the follow up phase (Chapter X)

The first but frequently overlooked sign of left sided heart failure is pulmonary venous dilatation following pulmonary venous hypertension (Short 1956 Simon 1961, Logue *et al* 1963) This is caused by increased filling pressure when the left ventricle (or atrium) fails as a pump and the end diastolic pressure rises in consequence (Lewis *et al* 1953) Hemodynamic studies (Lavender *et al* 1962) have shown that pulmonary venodilatation develops when the mean pressure of the left atrium rises above 10 mm Hg Clinically, this phase is altogether symptomless (Wood 1956) except for the radiological finding

Dilatation of the pulmonary veins occurs initially in the lower fields on which hydrostatic pressure also exerts an effect, and gradually also in the upper fields whereupon only the normally inconspicuous veins can be clearly distinguished from the pulmonary arteries In the study of Logue *et al* (1963) there was no venous dilatation in only 10 % of the patients when other clinical signs of left sided heart failure were present Under prolonged strain a vasoconstriction of the

lower fields gradually develops as a consequence of complex hydrodynamic and neurohumoral series of occurrences (West 1965 1966) The increased venous pressure gradually induces oozing of fluid into the pulmonary tissue and the interstitial edema gives a hazy, clouded appearance to the perivascular areas and the whole pulmonary tissue This is often attributed merely to a poor radiological technique

When the pulmonary capillary pressure rises above 18—20 mm Hg the septal lines described by Kerley (1933) appear (Grainger 1958) They are induced by the edema and the fibrous thickening in the pulmonary lobular septa Dilatation of the pulmonary artery and its main branches occurs gradually with increasing pulmonary pressure Intra alveolar edema, with its pronounced radiological changes (Jackson 1951) is the last and well known step in the pulmonary changes produced by left sided heart failure

The present study revealed that prominent pulmonary signs of left sided heart failure can often be detected even when the heart has not been enlarged, as also emphazised by Zatuchni and Nussbaum 1963, Logue and Hurst 1966a) Logue *et al* (1963) reported that in its initial stages left sided heart failure could be much more sensitively perceived radiologically than clinically, in one fourth of the patients subsequently confirmed left sided heart failure was first noticed in radiological examination only

Evidence was cited earlier (p 61) that left-sided heart failure appears to be almost universally present in acute myocardial infarction, as revealed by pulmonary function studies and blood gas analysis In the present study, the results of clinical findings and hemodynamic investigations carried out on follow up (Table 19), agreed well with the common occurrence of the radiological signs of left sided heart failure

Equal incidence of aortic sclerosis in both patient groups does not support the view that a murmur of aortic sclerosis would in fact have been interpreted erroneously as an atypical ejection type mitral systolic murmur (Chapter VI)

To sum up 55 % of the patients in whom mitral incompetence developed in connection with myocardial infarction had normal heart volume This refutes the common assumption that the mechanism of murmur is secondary to mitral annular distention The size of the left atrium does not enlarge acutely even under a severe hemodynamic burden Signs of pulmonary venous congestion after acute myocardial infarction were extremely common even with a normal size of heart, and more so in patients with acute mitral incompetence

HEMODYNAMIC INVESTIGATION

PATIENTS

Twenty five patients were succesfully obtained for voluntary inclusion in the hemodynamic investigation

Marked mitral incompetence was estimated to be present in 6 patients In 5 of the 6 patients the condition was judged to be marked on clinical grounds but the original estimation of somewhat slight mitral incompetence in one patient had to be revised efter cardiac catheterization study This patient had only a grade 1 apical pansystolic murmur but a very hyperdynamic left ventricular impulse

Slight mitral incompetence was present clinically in 12 patients, and was confirmed by hemodynamic study

No murmur patients 7 patients were examined 6 from the clinical no-murmur group yielded no signs of mitral incompetence but were found to have congestive heart failure varying by the N Y H A criteria from grade I to IV The seventh patient had had a mitral systolic murmur in hospital that was no longer present during the follow up study when the catheterization was performed

METHODS

ESTIMATION OF SEVERITY OF MITRAL INCOMPETENCE

It is generally agreed that no single method can reliably and accurately measure quantitatively the mitral regurgitation in an individual patient despite various attempts made with radiological hemodynamic and dye dilution techniques in addition to clinical evaluation (Shillingford 1962) Of these methods left ventricular high-speed cinecardioangiography obviously most nearly approaches the desired standard of quantitative discrimination (Ross and Criley 1962 Rees *et al* 1965 Logan *et al* 1967) The left atrial direct or indirect pulmonary capillary wedge pressure curve (PCW) often accomplishes a rough gradation of the regurgitant volume (Lagerlof and Werko 1949 Dexter *et al* 1950 Wade *et al* 1952 Gorlin *et al* 1952, Kent *et al* 1954 Wells 1958, although errors are not uncommon (Wiggers 1953 Ross *et al* 1958 1960 Braunwald and Awe 1963)

In the present investigation, right heart catheterization was chosen as a safe method for the hemodynamic study of patients in the convalescent stage of myocardial infarction

In order to improve the accuracy of the method a pharmacodynamic methoxamine test was applied This method has repeatedly been demonstrated as also giving sensitive recognition of a slight degree of mitral regurgitation.

For lack of an efficient cine-cardioangiographic apparatus it was not considered feasible to use left ventriculography in patients with ischemic heart disease and only slight mitral incompetence or none at all

All the cardiac catheterization studies were carried out by the author

PRINCIPLES OF THE LEFT ATRIAL PRESSURE CURVE ALTERATIONS

Blood regurgitating from the left ventricle in the low-capacity left atrium and pulmonary veins usually produces characteristic changes in the pressure of the left atrium. These can be studied indirectly from the pressure curve obtained by wedging the catheter into a peripheral branch of the pulmonary artery (Lagerlof and Werko 1949)

Alteration of the pressure curve can range through all the degrees related to the amount of regurgitation from almost normal to a gradual levelling-off of the *x* descent and a heightening of the *v* peak so as eventually to approach the form of the ventricular pressure curve (Hamer *et al* 1959 Ross *et al* 1960 Werko 1962 Kaplan 1966)

An increased systolic distention of the left atrium and a rapid unobstructed diastolic flow through the atrioventricular orifice steepen the *y* descent of

o d f ll ng phase of the left ventr cle (W ggers d F l 1922 Lagerlof and Werko 1949 Gorl n 9 ? Nixon and Wooler 1963 b) Var ous d ed f om the left atrial pressure levels he eloc y of their change have been a y correla ed to the amount of the ow Owen and Wood 1955 Morrow The lef entricular diastol c pressure o rise consp cuously because the d stended le l ke the atr um expels the ncrea ed f b ood by improved eff c ency (Jones o p ov ded . competence of the left h no developed (Ross et a 1958)

NC PLE OF THE PHARMACODYNAMIC TEST

Res s an e to he forward flow from the left ricle s one mportant determinant of the un of th regurg tant stroke volume through a leak ng m tral val e (W ggers and Fe l 1922 Rodbard and W ll ams 1954 Da ila 1958 Braun ald t al 195 Jose et al 1964)

The effect of pressor agents in augment ng n tral regurgi at on by rising per pheral system c ar erial resi ance has been demonstrated by ex ernal and ntracardiac phonocard ography Endrys and Bártova 1962 Perloff and Harvey 1962 Leda et al 1966 Leighton et al 1966) by d rect and indirect left atr al pressures (Braun ald et a 19 8 Stanfield and Yu 1960) and by the dye d lut on techn que (Yu et al 1961 Rutis hauser et al 1963 Tanenbaum and Pfaff 1963 Jose et al 1964) The normal left atr al pressure curve occas onally present in sl ght mitral in competence is usually unmistakably changed by pressor agents to that of mitral ncompetence Ross et al 1960 Le ghton et al 1966

Methoxam ne as used in the present study because t ncreases sys em c arte al res stance and consequently blood pressure bout any notrop c effect on he myocard um n add t on to wh h t has no effect on the pulmonary arter al pressure or pulmonary vasoconstrict on Ross et al 1960 Yu et al 1961 Tanenbaum and Pfaff 1963 Ueda et al 1966) The effect was registered simul aneously by bo h pulmonary cap llary edge pressure and external phonocard ography An ndex of o er 35 % of the ncrement of the peak d ded by the increment of sys ol c arter al press re has usually been reported to ndica e the presen e of hemodynam cally-s gn ficant mitral n ompe ence S anfield and Yu 1960)

T murmur of m tral incompe ence increases in ns ty w th ncreased regurgitant flow Ir n aort c and systol c eject on murmurs de ca e n ntens ty (Lu sada 1965 Ueda et al 1966

ESTIMATION OF OXYGEN CONSUMPTION

To dete n ne he oxygen consumption exp red air was collec ed f r seven m nutes by means of an a r t ght mask a low res stance valve system and tub ng into a Douglas bag The collected a r in the bag was empt ed by fold ng nto a dry gasometer The volume of O_2 consumpt on was calculated from the oxygen content of the exp red a r and the room a r

OXYGEN SATURATION AND CONTENT OF BLOOD SAMPLES

The oxygen saturat on of blood samples was determ ned with an Elema oxymeter The hemoglob n was analyzed as oxyhemoglob n by the cyanmethemoglob n techn que The oxygen content of each sample was determ ned by a nomogram us ng a hemoglob n concentrat on and an oxygen saturat on The normal arterio venous oxygen d fference at rest var es bet een 3 and 5 volume % (Holmgren et al 1957) slowly increas ng in older age groups (Granath et al 1964)

CARDIAC OUTPUT

The direct Fick pr nc ple of calculat ng the cardiac output from the oxygen consumpt on and arter o enous oxygen d fference was appl ed and the result expressed as a card ac index The normal card ac ndex var es between 3 and 4 5 l /min/m² of BSA (Dexter et a 1950 Wade and Bishop 1962 Bayer et al 1967) In this labor atory the normal values var ed from 2 7 to 6 1 mean 4 6 l min/m² of BSA

PRESSURE RECORDING

Equ pment Cournand type woven nylon USCI s ngle lumen end hole ca heters of s ze 7 F or disposable s ze 6 F were used in the r ght s de cardiac catheterizat on Pressures were transduced by the electr cal Sanborn pressure transducer 267 B hich was connected to a Carr er pream pl fier 350 3 000 and recorded by a 4 channel Sanborn polybeam record ng system (568) w th opt cal gal anometers on photograph c film. A rap d developer attached to the recorder made vis ble recordings ava lable instantaneously

Record ng An undamped record ng was usually used and only occasionally was a damp ng system used to reduce 30 40 cps catheter resonance The electrocardiogram lead II external phonocard ogram intravascular pressure curve and pressu e zero-level were recorded s multaneously Full s ale sens t vity was generally used n the pressure record ng The film speed was 25 mm/sec n n rarard ac pressures and 100 mm sec when recording pulmonary cap llary edge (PCW) pressure Electr cal damp ng was used to obta n mean pressures The cal brat on was registered after each record ng both electrically and hydrostat cally The zero-level stab l ty was always checked before and after each pressure recording The zero level used was 10 cm above table level

The normal levels in this laboratory were compared with those stated by others (Kaplan 1966) PCW systolic 8—14 diastolic 4—8 mean 6—11, pulmonary artery systolic under 30 right ventricular diastolic under 7, right atrial mean under 8

Phonocardiograph equipment A crystal contact type, high impedence Sanborn 350—1 700-C 10 microphone with a frequency response of over 1000 cps was used, connected to a heart sound preamplifier 350—1700 B The following octave related high pass filters were used, mostly 200 cps band flat ± 3 db between 200 cps and 1 KC with a damping of 24 db at 100 cps and 60 db at 25 cps A 400 cps band was sometimes used to detect high frequency murmurs with greater sensitivity but often the inappropriate noise signal ratio obtained with this high frequency band necessitated changing over to a 200 cps cut-off band to enhance clarity

Procedure Right sided cardiac catheterization was performed in the morning with the patient in a state of fasting following premedication with 25 mg promethazine chloride Phenergan ® A heart sound microphone was strapped to the point of maximal murmur intensity With the help of an oscilloscope appropriate filter and amplification were selected and maintained without further change A catheter was introduced into the right or the left basilic vein and advanced into the heart under television control Blood samples were withdrawn from the superior and inferior caval veins and from the right atrium to exclude shunts At least three blood samples were drawn from the pulmonary artery trunk for arteriovenous oxygen difference calculation The brachial artery was punctured by a hypodermic needle for arterial blood sample and pressure recording

The pressures in the right atrium right ventricle and pulmonary artery were recorded before wedging the catheter into the peripheral branch of the right or the left pulmonary artery When a soft number 6 F disposable catheter was used it was sometimes necessary to stiffen it with metal guide wire as used in the percutaneous Seldinger technique (1953) before adequate positioning in the wedging was achieved

After the basal PCW pressure level had been registered injections measuring 1—2 mg at a time of methoxamine chloride Vasoxine ®, Burroughs Wellcome & Co 20 mg having being diluted in 20 ml of physiological saline were given through the catheter Generally after a 3—4 mg injection a response was obtained consisting of a rise in systolic arterial pressure of not less than 10 mm Hg as measured repeatedly by brachial cuff and bradycardia Patients complained at the same time of slight feeling of pressure chill, goose flesh or a need to void which was helpful in determining the effect and dose of the drug Immediately after these signs the PCW pressure and phonocardiogram were recorded in the same way as the previous control curve the blood pressure being measured immediately after the PCW recording by cuff or often also by an intravascular pressure recording The effect of the drug was very transient lasting only 2 or 3 minutes In most patients repetition of the pressor test after some ten minutes yielded a reproducible response in PCW pressure Oxygen consumption was determined after the pressure recordings and was thus not quite simultaneous with the blood sampling

COMMENTS

CARDIAC OUTPUT DETERMINATION

No determination was made of the methodical error of oxygen consumption in the present study but in similar systems it has been shown to correspond to a coefficient of variation within a few per cent (Granath 1965) The error of a single determination of blood oxygen saturation was 2 4 % and the error of hemoglobin concentration determination 0 6 % determined from successively withdrawn samples The methodical error of direct Fick cardiac-output determination usually falls within 10 % (Wade and Bishop 1962 Werko 1964) This includes both the analytical error and biological variations of the subject examined

The basal state is essential for cardiac output determination in conditions of right-sided heart catheterization the error arising from this factor has been observed to be within 20 % (Wade and Bishop 1962 Granath 1965 Bevegård *et al* 1966)

PRESSURE MEASUREMENTS

Pulmonary capillary wedge pressure and simultaneously recorded external phonocardiographic tracings are markedly affected by respiration (Kaplan 1966) Therefore the recordings were always carried out similarly at end-expiration respiratory apnea. The characteristic valid form of pulmonary capillary pressure curve has been reported as being obtained in between about 70 and 90 % of the patients (Werko 1964) In the present series at least slight heart failure was present in most of the patients and by distending the *left atrium and the pulmonary venous channels* it probably facilitated obtaining valid PCW curves (Ross *et al* 1960 Braunwald and Awe 1963 Kaplan 1966) The form of the PCW pressure curve was monitored on the oscilloscope with full scale sensitivity and if the curve did not prove representative another pulmonary arterial branch was wedged (Bell *et al* 1962)

Varying statements about the validity of the pulmonary capillary wedge pressure curve in reflecting the left atrial pressure curve have been made but it has generally been recognized as being quite closely related to the left atrium pressure wave form and level in man provided

unrepresentative tracings are excluded (reviewed by Werko 1962 1964 and Kaplan 1966)

The uniform dynamic response obtained with commonly used catheter systems has been shown usually not to exceed 8–20 cps (Fry *et al* 1957 Piemme 1963) However a uniform dynamic response of up to 5–8 cps has been considered to be adequate when only systolic and diastolic pressures without rapid rates of change are measured (Ellis *et al* 1951 Wood *et al* 1954) Careful debubbling of the pressure chambers was routine procedure in obtaining undamped curves Non linearity of the recording system on a full scale was not over 2 %

COMPLICATIONS

One patient (No 67) developed an RBBB during the course of catheterization This patient had had intermittent RBBB during hospital treatment of the acute phase of myocardial infarction. Another patient with marked regurgitation developed pulmonary edema after a dose of 3 mg methoxamine A good response was achieved by routine treatment

Table 20 *Hemodynamic data at rest in 25 patients no murmur 7 MIMI slight 12 MIMI marked 6*

	Mean	S D	P
Oxygen consumption ml/min			
No murmur	225	33	
MIMI slight	252	33	
MIMI marked	311	54	< 0 01
A – V O_2 difference vol %			
No murmur	4 8	1 3	
MIMI slight	4 8	1 0	
MIMI marked	6 7	1 2	< 0 01
Cardiac index l/min/m² of BSA			
No murmur	2 9	1 3	
MIMI slight	3 0	0 8	
MIMI marked	2 6	0 5	N S
Forward stroke volume ml/min			
No murmur	79	35	
MIMI slight	84	29	
MIMI marked	48	17	N S
Pulmonary vascular resistance units			
No murmur	1 3	0 9	
MIMI slight	1 3	0 7	
MIMI marked	2 4	1 4	N S
Right atrial mean pressure mm Hg			
No murmur	5	1	
MIMI slight	5	3	
MIMI marked	7	5	N S
Pulmonary artery mean pressure mm Hg			
No murmur	16	2	
MIMI slight	19	6	
MIMI marked	38	13	< 0 001

N S non significant

P values are based on F test

RESULTS

HEMODYNAMICS AT REST

The results of the hemodynamic investigation of patients with slight or marked MIMI as compared with the results obtained in the no-murmur group are presented in Tables 20 and 21 and in Figs 36 and 37

It is evident that the [redacted] patients with marked mitral incompetence suffered from much more severe circulatory disturbances than the others The hemodynamic alterations in the group of patients with slight mitral incompetence varied but were mainly comparable to the degree of the disturbance resulting from heart failure, observed in patients without mitral incompetence

Heart rate, oxygen consumption arteriovenous oxygen difference, pulmonary artery and wedge pressures, differed significantly or highly significantly in the variance test when the marked MIMI group was compared with the groups of patients with slight or no mitral incompetence

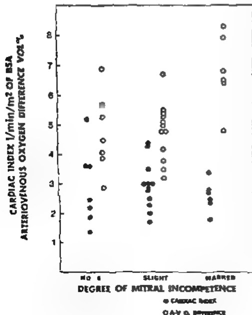


Fig 36 *Cardiac index and arteriovenous oxygen difference obtained at cardiac catheterization.*

The values of systolic arterial pressure, cardiac index, forward stroke volume, pulmonary vascular resistance and right atrial pressure also tended to deviate more from the normal in the marked MIMI group than in the others, although statistically not significantly

The arterial blood oxygen saturation was below normal, 96 %, in 11 patients in all but one patients in the marked MIMI group, and in 3 patients in the slight MIMI and 3 in no-murmur groups In no patient did the form of the right atrial pressure curve reveal any signs of tricuspid incompetence The mean right atrial pressure was increased abnormally in only 4 patients The pulmonary arterial systolic pressure exceeded 30 mm Hg in 4 patients in the marked mitral incompetence group, in 4 patients with slight mitral incompetence and in 1 patient without mitral systolic murmur

The form of the pulmonary capillary wedge pressure (Table 22) was abnormal at rest, suggestive of mitral incompetence in all the 6 patients in the marked mitral incompetence group but in only 3 patients in the slight mitral incompetence group In 8 patients with slight MIMI, the form of the pulmonary capillary wedge pressure curve could not be differentiated from the normal, while in 1 patient (No 43) it was uncharacteristic of the left atrial pressure curve and invariably similar though taken from several pulmonary artery branches In all the no-murmur patients the PCW form was normal though its pressure level might be high

It is evident from the Table 21 and Fig 37 that in the marked mitral incompetence group the pressures were generally highly abnormal, but in several patients with slight mitral incompetence, the range was about the same as in patients without mitral incompetence It is noteworthy

Table 21 *Pressor response in pulmonary capillary wedge pressure at methoxamine test in 25 patients, no murmur 7 MIMI slight 12 MIMI marked 6*

	CONTROL			METHOXAMINE		
	Mean	SD	P	Mean	SD	P
Systolic systemic arterial pressure mm Hg						
No murmur	129	29		153	35	
MIMI slight	150	26		174	20	
MIMI marked	139	37	N S	154	42	N S
Heart rate beats/min						
No murmur	69	21		52	14	
MIMI slight	69	11		58	11	
MIMI marked	94	16	< 0.01	93	22	< 0.001
a wave mm Hg						
No murmur	15	4		20	5	
MIMI slight	16	5		22	7	
MIMI marked	32	9	< 0.001	39	7	< 0.001
v peak, mm Hg						
No murmur	14	4		14	5	
MIMI slight	15	6		15	10	
MIMI marked	34	15	< 0.001	36	17	< 0.001
y trough mm Hg						
No murmur	9	4		14	4	
MIMI slight	10	4		15	4	
MIMI marked	19	8	< 0.01	25	8	< 0.001
PCW mean mm Hg						
No murmur	10	3		15	4	
MIMI slight	11	4		16	5	
MIMI marked	26	8	< 0.001	34	9	< 0.001
Ry/v						
No murmur	2.1	0.7		1.7	0.5	
MIMI slight	2.0	0.9		1.9	0.8	
MIMI marked	4.0	0.8	< 0.001	5.0	1.1	< 0.001
Ry/meanPCW						
No murmur	3.0	0.9		2.3	0.9	
MIMI slight	2.5	0.9		2.8	1.3	
MIMI marked	5.9	1.5	< 0.001	7.6	1.8	< 0.001

N S non-significant
P values are based on F test

that though the pressures among the no-murmur patients had also slightly or, in two cases, appreciably risen compared to the normal values, the form of the pressure curve nevertheless remained normal No ventricularization type form of pulmonary capillary wedge pressure curve was ever detected at rest, but one appeared after a methoxamine test (Fig 38) Although the *v* peak was apt to reach the extreme

Fig 37 *Values of v peaks, y troughs and means of the pulmonary capillary wedge pressure curves and their response to methoxamine in patients with and without mitral incompetence*

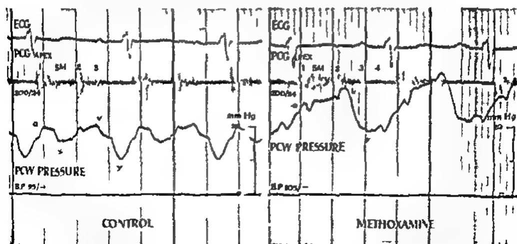


Fig 38 *Ventricularization type response in the pulmonary capillary wedge pressure induced by methoxamine Increased mitral regurgitant flow also resulted in louder mitral systolic murmur The rise of the systolic arterial pressure was only slight Film speed 100 mm/sec*

high of 40—60 mm Hg, it did not appear until rather late in systole (Fig 39)

Indices of the PCW pressure curve The ratio of the velocity of the *y* descent to the *v* peak, Ry/v, and to the mean PCW pressure, Ry/meanPCW, was highly abnormal in all the patients in the marked mitral incompetence group and normal in all the members of the no-murmur group In the patients with slight incompetence there was some variation between pathological and normal values Generally, however, they were normal in this group, there being only 5 exceptions

Murmur intensity and type related to peak v wave pressure No definite correlation with the height of the *v* peak and the intensity of the mitral systolic murmur was observed (Fig 40) For example, in the marked incompetence group, there was one patient with only a grade 1 plateau-type murmur and a *v* peak of 25 mm Hg, while in another with a grade 1—2 crescendo murmur, the *v* peak was 48 mm Hg (Fig 41), and otherwise one patient with a grade 4 murmur had a *v* peak of 20 mm Hg All the murmurs in the marked MIMI group registered a pansystolic plateau or crescendo type, while in the members of the slight MIMI group all the four subtypes of pansystolic murmur — plateau, crescendo, decrescendo and crescendo-decrescendo — were observed

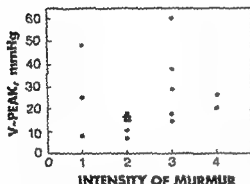


Fig 40 Relation of the height of the v peak to the intensity of the mitral systolic murmur

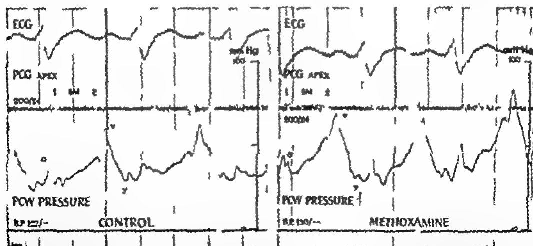


Fig 39 *The pulmonary capillary wedge pressure tracing shows a highly peaked late v wave further intensified by administration of methoxamine The rise of the diastolic pressure level by methoxamine was insignificant but the increased mitral regurgitant flow precipitated an acute pulmonary edema Film speed 100 mm/sec*

PHARMACODYNAMIC TEST

The heart rate (Table 21) was initially high (94) in marked mitral incompetence group and tended to slow down only insignificantly (93) after a methoxamine injection, in 2 patients it rose, the mean change being only -13 ± 15.9 %

In patients with a slight incompetence or none at all, the heart rate invariably slowed down, the mean change being -13.5 ± 8.4 % and -24.1 ± 10.7 % respectively The differences were significant ($p<0.01$) No ectopic beats were observed as being produced by methoxamine

The rise in systolic *arterial pressure*, (Table 21) again, was slightly lower in patients with marked mitral incompetence as compared to the patients with slight or no mitral incompetence These differences however, were not significant The increase was 11.2 ± 5.3 % in the marked mitral incompetence group, 16.8 ± 6.1 % in the group with slight incompetence, and 18.4 ± 4.8 % in the no-murmur group

The arteriovenous oxygen difference was not determined systematically, but it increased in all the cases examined while the methoxamine maintained its effect

Pulmonary capillary wedge pressures (Table 21, Fig 37) The *a* wave rose slightly in all the patients, however, highly significantly in the marked MIMI group

The rise in the *v* peak was appreciable in the patients with marked mitral incompetence In one patient the *v* peak rose from 60 to 86 mm Hg in response to a small methoxamine dose (Fig 39), this precipitated an immediate pulmonary edema which responded rapidly to the usual treatment In patients in the slight mitral incompetence group, the rise was less steep

In patients without any murmur, the rise in the *v* peak was slightest and concomitant with the increase of the whole PCW pressure level

The rise in the *y* level, presumably reflecting a myocardial failure, was not so conspicuous ($p<0.01$) as the rise in the *v* peak ($p<0.001$) among the patients in the marked mitral incompetence group when compared with the other groups (Fig 37)

The change in PCW mean pressure was similar to that in the *y* level

The ratio of the increment of the *v* peak to the increment of systolic arterial pressure, $\Delta v/\Delta A$, was invariably pathological in the marked incompetence group ranging from 70 to 250 %, mean 128 % (Fig 42) In the group with slight incompetence, it ranged from 7 to 113 % the mean being 46 % In 5 of the 11 patients (in 1 patient the test failed for

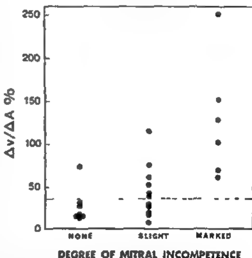


Fig 42 *Ratio of the increment of v peak to the increment of systolic arterial pressure ($\Delta v/\Delta A$) in the methoxamine test Dotted line represents the upper limit of the normal reported by Stanfield and Yu (1960)*

technical reasons), it fell to a normal range below 35 % (Stanfield and Yu 1960)

Among all but one of the no-murmur patients the ratio was normal (mean 29%), the single exception registering a value of 67 % This patient had marked heart failure and the whole PCW pressure level

Table 22 *Influence of the pharmacodynamic test on the form of the pulmonary capillary pressure (PCW) curve and on mitral systolic murmur*

CASE NUMBER	PRESSURE TRACING: Form of the PCW pressure curve consistent with mitral incompetence — Control	PRESSURE TRACING: Form of the PCW pressure curve consistent with mitral incompetence — Enhanced by methoxamine	PHONOCARDIOGRAPHY: Murmur intensity enhanced by methoxamine	AUSCULTATION: Character of the murmur — Grade	AUSCULTATION: Character of the murmur — Type
			Marked mitral incompetence		
51	(+)	+	+	2–3	Plateau
67	+	+	+	0–1	Plateau
121	+	+	+	3–4	Crescendo
95	(+)	+	+	4	Plateau-crescendo
99	+	+	+	4	Plateau
84	+	+	+	1–2	Crescendo
			Slight mitral incompetence		
59	(+)	+	(+)	3	Crescendo-decrescendo
116	(+)	+	+	2	Plateau
43	–	–	–	3	Crescendo decrescendo
93	–	(+)	+	2	Plateau
40	–	+	+	2	Decrescendo
96	(+)	+	–	2	Decrescendo
41	–	(+)	(+)	1	Decrescendo
47	–	–	–	2	Decrescendo
85	–	–	(+)	2	Decrescendo
50	–	(+)	(+)	2–3	Decrescendo
92	–	(+)	+	2	Ejection-decrescendo
52	–		(+)	2	Plateau
			No mitral incompetence		
139	–	–			No murmur
140	–	–			No murmur
167	–	–			No murmur
44	–	–			No murmur
141	–	–			No murmur
161	–	–			No murmur
136	–	–			No murmur

+ marked
(+) slight
– none

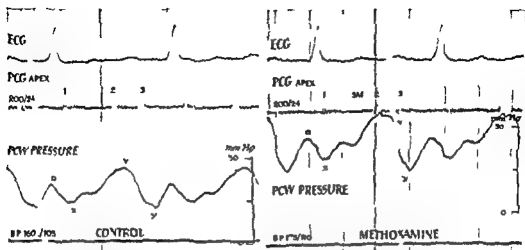


Fig 41 *Considerable alteration of the pulmonary capillary wedge pressure curve to the pattern of mitral incompetence in a patient with only a grade 1—2 high frequecy crescendo pansystolic murmur (Fig 35) The murmur is barely visible in an external phonocardiography under the effect of methoxamine Note loud third heart sound Film speed 100 mm/sec*

rose noticeably without causing the definitely normal form of the PCW curve to disappear

The indices of the pressure curve, Ry/p and Ry/mean PCW, increased among the patients in the mitral incompetence group, though slightly decreasing in 2 patients while among the no-murmur patients it decreased, with only 1 increase being registered

Form of the pulmonary capillary wedge pressure curve (Table 22) The characteristic form of the pressure curve observed in patients with mitral incompetence was invariably intensified by methoxamine in the marked mitral incompetence group (*Fig 43*) In the no murmur group the normal form of the curve occasionally became more clear cut as the amplitudes rose slightly but in no instance did it deviate from the normal form in the direction of mitral incompetence by a rise in the *v* peak in relation to the *a* peak or by a rise in the *x* level (Fig 44) By contrast, in the slight mitral incompetence group the initial tracing indicating mitral incompetence was further augmented in all 3 patients while in 5 patients with mitral systolic murmur exhibiting the normal form of pressure curve at rest, it changed to one indicating mitral incompetence only after a methoxamine infusion (Figs 45 and 46) In 3 patients included in the slight MIMI group no definite change occurred in the normal PCW pressure curve apart from a general rise in the pressure level

The phonocardiogram tracings (Table 22) showed increased murmur intensity in all the 6 patients with marked mitral incompetence and in 9 of the 12 patients in the slight mitral incompetence group (Figs 38, 39 41 43) 3 patients in the latter group developed no observable intensification of the apical systolic murmur However, one of them did have a typical PCW pressure curve consistent with mitral incompetence as further enhanced by methoxamine One patient (No 43) registered an uncharacteristic PCW curve, but on the third day after the onset of the infarction he had developed a constant grade 3—4 apical pansystolic murmur, and fluoroscopy revealed a rapid early

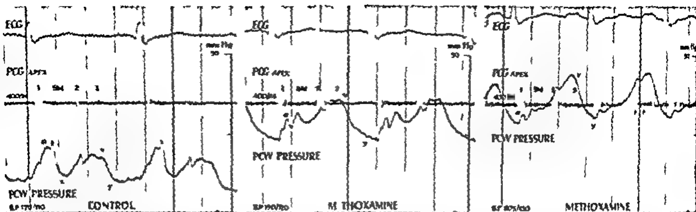


Fig 43 Methoxamine induced alterations suggestive of mitral incompetence, observed in external phonocardiography and in a pulmonary capillary wedge pressure (PCW) curve The murmur intensity was markedly increased decrescendo type in the control tracing was changed to plateau-crescendo with an extension of the murmur over second heart sound (arrows) Initially the completely normal PCW curve lost the x descent and developed a high v peak and a steepened y descent, changes being clearer with increasing dose of the drug A conspicuously elevated diastolic pressure level was interpreted to indicate acute left ventricular failure Film speed 100 mm/sec

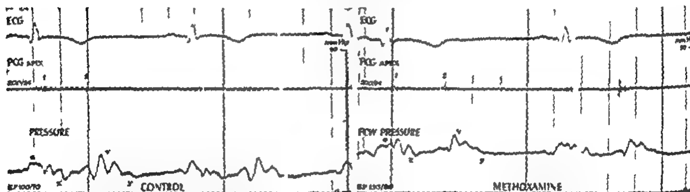


Fig 44 Pulmonary capillary pressure in a patient without mitral systolic murmur The methoxamine administration did not change the normal form of the curve The reflex bradycardia was marked Film speed 100 mm/sec

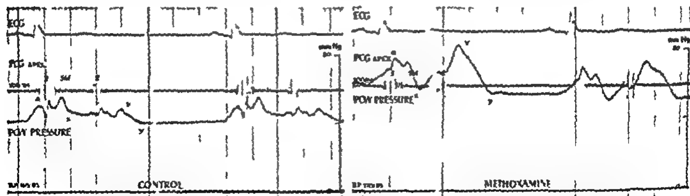


Fig 45 Change of the normal pulmonary capillary pressure curve to the pattern suggestive of mitral incompetence (heightening of the v peak) by methoxamine in a patient with a slight mitral incompetence Film speed 100 mm/sec

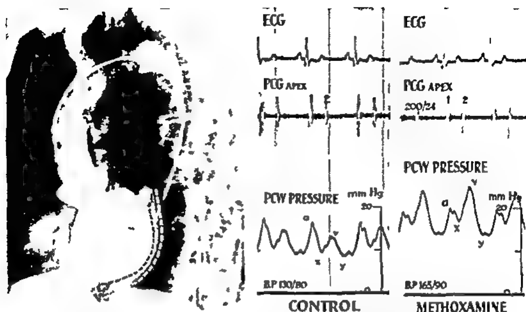


Fig 46 *Left ventricular cardioangiography reveals the very thin posterior wall of the left ventricle (dotted lines) A marked paradoxical pulsation was present (Fig 23 the last tracing) The pharmacodynamic test resulted in a clear elevation of the v peak characteristic of mitral incompetence Film speed 25 mm/sec*

systolic expansion of considerable amplitude of the left atrium Mitral incompetence thus seemed probable Another patient (No 47) who exhibited no changes of pressure or murmur intensity in the pressor amine test had developed a constant apical pansystolic high frequency grade 3 murmur on the 16th day after the onset of infarction, thus the only finding that pointed to mitral incompetence was the characteristic murmur

Left ventricular cardioangiography For reasons explained earlier (Methods) left ventricular angiography was performed only once Right sided catheterization had confirmed clinically slight to moderate mitral incompetence in this patient The left ventricle was entered retrogradely via the femoral artery by application of the Seldinger (1953) technique Pictures were taken in the right oblique position using the serial exchanger with a maximum speed of 6 frames a second No visible regurgitant jet was observed but the posterior wall of the myocardium was conspicuously thinned (Fig 46) and the posterior mitral leaflet seemed to bulge toward the left atrium during the systole

DISCUSSION

Degree of hemodynamic changes Evidence of a markedly varying degree of mitral incompetence could be obtained in 16 of the 18 MIMI patients included in the hemodynamic investigation This result was accomplished by means of a pulmonary capillary wedge pressure curve determination and of pharmacodynamic phonocardiography Changes characteristic of mitral incompetence were disclosed in 6 patients only by the infusion of methoxamine which augmented the regurgitation (Leighton *et al* 1966)

The results of the hemodynamic study at rest showed that in all 6 patients classified as having a marked regurgitation the hemodynamic disturbance was fairly

severe The manifestations were a clearly altered PCW pressure curve and unequivocal signs of left-sided heart failure, hyperventilation, tachycardia, an increased arteriovenous oxygen difference, a lowered or low normal forward cardiac index, and a conspicuously depressed forward stroke volume The pulmonary vascular resistance was not generally raised, reflecting a rise in the observed elevated pulmonary artery pressure, to have been induced passively by the regurgitation The signs of right sided heart failure were rare

In contrast, in patients with only slight mitral incompetence, the hemodynamic changes varied in degree and proved mostly to be minor ones, approaching the findings observed in patients without mitral incompetence

The patients without mitral incompetence were characterized by changes consistent with left sided heart failure of varying degrees

Pulmonary capillary wedge pressure curves in patients with clinically marked mitral incompetence were characteristic of significant mitral regurgitation Although the *v* peaks were often high, they occurred late in systole, with a normal *x* descent position (Nixon and Wagner 1962) Only once was a ventricularization type of pressure curve observed after a pressor amine test Raftery *et al* (1966), in their acute subvalvar mitral incompetence series, noted the same phenomenon, and their explanation of "tight incompetence" with severe regurgitation to a normal sized uncompliable left atrium is evidently also in agreement with the present investigation

A low level of *v* peak does not always indicate slight regurgitation, in that compliance of the left atrium and its pressure-volume characteristics can definitely alter the response (Ross *et al* 1960, Braunwald and Awe 1963, Werko 1964 Raftery *et al* 1966) At low atrial pressure levels, the *v* peak may be only insensitively altered by a substantial regurgitant flow At an increased mean pressure level, the rise in the *v* peak caused by regurgitation appears more readily *V* peaks exceeding *a* waves have been reported as occurring in left ventricular failure or pulmonary hypertension without evidence of mitral incompetence (Lagerlof and Werkö 1949, Eliasch 1952) Otherwise, Jacono *et al* (1961) observed signs of acute mitral incompetence in experimental pulmonary edema precipitated by a rise in systemic arterial pressure

In the present study, *v*-wave changes were associated with the murmur of mitral incompetence, both of which usually were altered characteristically by methoxamine In no murmur patients with marked heart failure, oscillations of the *a* and *v* peaks exceeded the normal but did not have any shallowing of the *x* descent or peaking of the *v* wave substantially higher than the *a* wave (In this laboratory direct or indirect left atrial pressure curves simulating mitral incompetence have not been encountered in patients studied for aortic valve disease if mitral incompetence actually could not be demonstrated)

These inaccuracies of pressure curve analysis again emphatically illustrate the value of an effective left ventricular cinecardioangiography in the study of the mode and grade of the mitral regurgitant flow

The level of the *y* trough, reflecting left ventricular diastolic pressure, was generally above normal This is generally assumed to be a sign of left ventricular failure, although other factors involved in ventricular dynamics might elevate it (Braunwald and Ross 1963)

Methoxamine test The value of the methoxamine test in making slight mitral incompetence recognizable by increasing the intensity of the murmur and by inducing typical pressure curve alterations was clearly demonstrated in the present study when applied to patients, the level and form of whose initial pulmonary capillary wedge pressure curves were quite normal

By its lack of an inotropic effect, methoxamine by increasing the pressure load of the heart augments any underlying signs of heart failure (Harrison *et al* 1964). In the present study, a failing heart often increased the arterial blood pressure only slightly when peripheral resistance increased and, instead of reflex bradycardia, the acutely provoked heart failure resulted in tachycardia. This response was observed in certain patients with only marked mitral incompetence. Exceptionally, it is also apt to lead to erroneous interpretations of the methoxamine test as indicating mitral incompetence, when the left atrial pressure and the *v* peak rise noticeably in conjunction with only a slight rise in the systemic arterial pressure, if the form of the left atrial or PCW pressure curve is not simultaneously analyzed. Changes in the Ry/v and Ry/meanPCW ratios clearly differed in different directions in patients with and without mitral regurgitation.

The potential danger in using the pressor amine test, especially a drug without any inotropic effect, is related to the powerful way it increases the mitral regurgitant flow. This was observed in one patient who developed acute pulmonary edema after a small dose of methoxamine.

Murmur type and intensity. The murmurs in patients with hemodynamically marked mitral incompetence were of the pansystolic plateau or crescendo type, and in patients with slight regurgitation mainly of the plateau or decrescendo type (Table 22). This confirmed the earlier clinical impression (Chapter VI).

The poor correlation of the murmur intensity to the degree of regurgitation was remarkable. In two patients with marked incompetence the murmur intensities were only grade 1 and grade 1—2. This finding agrees with the importance of the stream velocity in the generation of a murmur (Bruns 1959, Rodbard 1964). Obviously, only a faint murmur is generated by the large regurgitant stroke volume ejected slowly through a widely gaping orifice (Schrire *et al* 1961). The velocity of the ejection is further retarded in MIMI patients by the reduced contractile speed of the damaged myocardium (Gleason and Braunwald 1964).

The dynamics of regurgitation. The determinants of the mitral regurgitant stroke volume are the size of the leaking opening, the contractility of the left ventricle, the systemic arterial resistance and the inertia of the blood (Wiggers and Feil 1922, Rodbard and Williams 1954, Braunwald *et al* 1957, 1958, Jose *et al* 1964).

In myocardial infarction, the waste of the contractile energy must be an important factor in augmenting mitral regurgitation. The regurgitant volume again adds a strain to the damaged myocardium and the consequent dilatation further impairs the ventricular function curve (Sarnoff and Berglund 1954, Case *et al* 1954). This series of events could be a dynamic counterpart to the structural mechanism of "mitral incompetence begetting mitral incompetence" proposed by Levy and Edwards (1962). This probable dynamic mechanism might accelerate the rapid, often fatal deterioration of the pumping capacity of the heart when acute significant mitral incompetence is superimposed on an acutely damaged failing left ventricle.

On the other hand, the remarkable tendency of the mitral incompetence brought on by ischemic damage to the muscular supporting structures of the mitral valve to lessen or disappear, was observed in the follow up study. This might also be attributed to a slow gain of better myocardial contractility after the acute phase had subsided. The related factors are probably a diminishing of the ischemic and edematous paralyzed area of the myocardium by the healing process and perhaps the development of collateral circulation (Chapter XI). These circumstances suggest that the condition of the wall of the left ventricle has con-

siderable significance in the mechanism of MIMI, in addition to the varying degree of anatomical destruction sustained by the papillary muscle itself

To sum up the hemodynamic severity of the mitral incompetence which develops after acute myocardial infarction can vary in degree from the barely detectable to the extremely severe even without rupture of the papillary muscle The severity of the regurgitation cannot be predicted from the intensity of the murmur Hemodynamic changes caused by the infarct *per se* add to the difficulty of estimating the degree of mitral incompetence The depressed function curve of the ischemic myocardium might be an important factor in determining the amount of regurgitation in addition to the structural destruction of the muscular support of the mitral valve itself

AUTOPSY FINDINGS

MATERIAL AND METHODS

MATERIAL

Out of the total of 187 patients 34 died in hospital In 30 of the cases it was possible to examine the heart at autopsy 19 of them had had an acute mitral systolic murmur, while the remaining 11 had had none One patient (No 190) with mitral incompetence that had developed during the previous infarction (group 3) was also examined The time of death was about the same in both groups and varied between 3 to [illegible] days after the onset of infarction the mean being 17 days The murmur of mitral incompetence had appeared at various times between the 1st and the 17th day, in 3 patients it was present on admission to hospital (see p 34)

One patient (No 51) who died one and half years after myocardial infarction and development of mitral incompetence was also examined at autopsy

METHODS

The grade of anatomical stenosis in the main trunks of the coronary arteries was determined by carefully opening them all the way to the periphery according to the technique presented by Hudson (1965) The following classification was used

0 — no changes
1 — stenosis of the lumen not filling half of it
2 — change obstructing 50 % or more of the lumen
3 — total occlusion

The site of any recent coronary occlusion was noted *The predominance of the coronary artery* distribution was determined according to the relation of the right coronary artery and the left circumflex branch [illegible] the crux of the heart as right balanced or left dominated (Schlesinger 1940)

The heart valves were then studied the atrioventricular valves via the opened atria and after cutting the apex cup transversally [illegible] determine the state of the mitral leaflets chordae tendinae and papillary muscles *in situ* before opening the heart further In order to determine accurately the site of the myocardial infarct, the heart was cut perpendicular to the long axis of the left ventricle into 5 or 6 transversal slices The site of recent and earlier infarction changes was ascertained and the size indices calculated from the measured length and width of the lesions The macroscopic state of the right and left ventricular papillary muscles was examined The mitral annulus was *measured with a conical device and again after* the annulus was cut open. The left and right ventricular wall thicknesses were measured Attention was further paid to the occurrence of mural thrombosis ventricular aneurysm mitral annulus calcification and of pericarditis.

After the macroscopic examination of the heart *both papillary muscles* were *fixed in formalin* and after processing embedded in paraffin The slides were prepared on 3 or 4 different transversal levels from the tip mid portion and base of both papillary muscles and stained by the van Gieson technique All hearts except one (No 4) were examined by the author The histologic preparations were examined without any knowledge of the name of the patient concerned. An experienced pathologist also studied the slides for evidence of necrosis in the papillary muscles

RESULTS

The results of the autopsy examination of the hearts are presented in Table 23 The following main findings and deductions were made

Table [redacted] Autopsy findings

Case number	Day of death	Coronary artery narrowing: Right	Coronary artery narrowing: Left circ	Coronary artery narrowing: Left ant desc	Site of recent coronary occlusion	Coronary artery predominance	Site of recent infarct		Size index of infarct	Extent of necrosis of papillary muscle: Anterior	Extent of necrosis of papillary muscle: Posterior	Heart weight g	Thickness of left/right ventricular wall mm	Circumference of mitral annulus mm	Disease of mitral valve	Calcification of mitral annulus	Aneurysm	Mural thrombosis	Pericarditis
Patients with mitral incompetence																			
1	7	3	2	2	Right	Right	Post sept + ant	UML	56	−	+	550	15/5	110	−	−	−	−	+
2	7	1	1	3	Left ant desc	Right	Anteroseptal	MI	35	+	−	440	13/6	108	−	−	−	+	−
3	45	2	2	1	Right + left circ	Right	Posterior	UM	35	-	+	470	15/6	110	−	−	−	−	−
4	11	0	0	2	Left ant desc		Anteroseptal	UML		+	+	720	25/5	norm	−	−	−	+	−
5	8	2	1	1	Right	Right	Posteroseptal	UML	56	−	+	580	20/5	112	−	−	−	−	−
6	47	2	3	3	Left ant desc + circ	Balanced	Anteroseptal	L	14	−	−	460	16/5	112	−	−	+	+	−
7a	4	2	0	3	Left ant desc		Anteroseptal	MI	20	+	−	410	14/5	95	−	+	−	+	−
8	C	3	3	2	Right	Right	Posteroseptal	UML	48	−	+	670	25/6	120	−	−	−	+	−
9a	3	3	2	3	Right + left ant desc	Right	Posterior + ant	UML	78	−	+	530	15/5	116	−	−	−	+	+
10	13	3	1	0	Right	Right	Posterior	UM	16	−	+	470	14/5	117	−	−	−	−	+
11	6	3	0	0	Right	Right	Posteroseptal	UM	30	−	+	415	14/5	100	−	−	−	−	+
12	16	1	2	2	Left ant desc + circ	Left	Posterior	UML	42	−	−	630	20/4		−	−	−	−	−
13	51	3	0	2	Right	Balanced	Anteroseptal	ML	36	−	−	810	26/5	115	−	−	−	+	−
14	16	3	1	1	Right	Right	Posterior	UML	64	−	+	585	20/5	128	−	−	−	−	−
15	4	2	2	2	Left ant desc	Balanced	Anterior	M	9	+	−	150	16/5	114	−	−	−	−	−
16	53	3	1	1	Right	Right	Anterior + post	UML	64	+	+	400	15/7	105	−	−	−	−	−
17	8	2	3	2	Left circ	Balanced	Posterior	UM	49	+	+	480	16/5	115	−	−	−	+	−
18	11	3	1	3	Left circ	Left	Lateral	UML	36	+	+	530	17/5	108	−	−	−	+	−
19	8	2	3	2	Left ant desc	Balanced	Anterolateral	L	40	+	−	390	15/4	90	−	−	+	+	+

Patients without mitral systolic murmur

20	10	0	0	3	Left ant desc	Right	Anteroseptal	ML	40	—	—	530	17/5	128	—	—	—	+	+
21	26	3	3	3	—	Right	Anteroseptal	ML	28	—	—	625	18/9	110	—	—	—	+	—
22	25	2	0	3	Left ant desc	Balanced	Anteroseptal	M	9	—	—	430	20 3	110	—	—	—	—	+
23	[illegible]	2	0	3	Left ant desc	Right	Anteroseptal	UM	16	—	—	460	13/5	110	—	—	—	—	—
24	30	0	0	3	Left ant desc	Balanced	Anterior	UMI	49	—	—	680	16 6	120	—	—	—	+	+
25	23	2	2	3	Left ant desc		Anterior	I		—	—	390	15/5		—	—	—	+	—
26	12	1	0	3	Left ant desc	Right	Anteroseptal	UMI	42	—	—	300	13/6	98	—	—	—	—	—
27	4	1	1	2	Left ant desc	Left	Anteroseptal	MI	20	—	—	490	18/5	110	—	—	—	—	—
28	3	3	3	1	—	Balanced	Posterolateral	M	6	—	—	140	17/5	109	—	—	—	—	—
29	15	1	1	3	Left ant desc	Balanced	Anteroseptal	UMI	61	—	—	580	17/6	110	—	—	—	+	+
30	30	0	0	3	Left ant desc	Left	Anteroseptal	UMI	63	—	—	460	14/5	118	—	—	+	+	—

U upper
M middle
L lower

Coronary artery stenosis In most cases, rather extensive changes in all the main trunks of the coronary arteries were observed In only 7 MIMI cases and 6 no-murmur cases, not more than grade 0 or 1 changes had occurred in the two major branches, apart from the branch with a recent occlusion

Cause of recent occlusion The most frequent cause of a recent coronary artery occlusion contributing probably to the development of the infarction, was a recent thrombosis superimposed on a small or large atheromatic alteration of the coronary artery wall A thrombotic occlusion was detected in 17 out of 19 cases of mitral incompetence while in the remaining 2 cases a totally occluding atheromatous change was detected In the no-murmur group a thrombosis was present in 6 cases, while in 3 cases a recent (?) atheromatous occlusion and in 2 other cases no recent change could be detected in the coronary arteries, where all the main trunks were severely stenosed

Predominance of coronary artery distribution A right coronary artery predominance was most common in the MIMI group 10 cases with a right predominance 5 with balanced circulation and 2 with a left predominance were found while in 2 other patients the predominance was not determined In the no-murmur group there were 4 cases with a right predominance, 4 registering a balanced distribution and 2 with a left predominance while one case remained undetermined

The site of the myocardial infarct was usually posterior in patients with mitral incompetence being observed in 12 cases (63 %), whereas it was anterior in only 7 cases (37 %) On the other hand the infarct was situated anteriorly in all but one of the 11 patients without any mitral systolic murmur

A posterior infarct in the MIMI group almost invariably involved the posterior papillary muscle to a serious degree

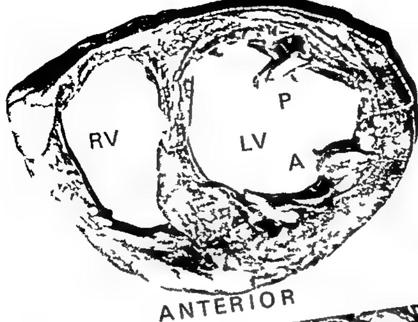
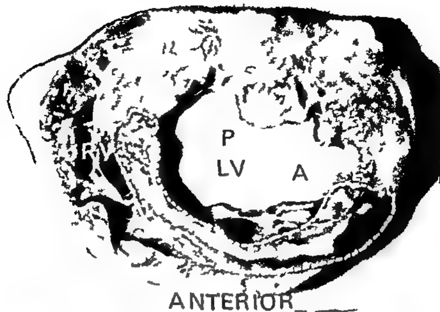






Fig 47 A se t n an er al plane from the heart of a pat ent w th an ac te m tral in ompetence follow ing myo ard al nfa t o The po te or pap llary muscle (P) is damaged by an extensi e posterior infarct Photom crog ap hs a lo power (3 x) and at greater magn ficat on (80 x) of the posterior pap llary muscle re eal an x n e de truct on with oagulat on ne ros s and nu lear debris (ran (e on) A anter or p p ll m s le RV r ght ventr cle LV left ventr cle







F g 48 *A se on n t an al plane f om the hea t of a pa ent u hout m al y tol c m rmur An extens ve an erio nfa t on has not rea hed the an e or pap llary mus le due to s ery la eral po tion n the left ven ricle A lou pouer (3 x) photom crog aph shows n olvement of only a small m d al segment (arrow) of the anterior pap llary mus le At g eater magn fi ation (80 x) only a patchy ne s of the muscle fibers was ev dent (an G e on) Fo abb eviat ons see F g 47*

(Fig 47), and when the infarct was anteriorly situated in the MIMI group the anterior papillary muscle was often significantly damaged In the no murmur group, although an anterior myocardial infarct might be quite extensive, it did not reach the anterior papillary muscle in 8 cases, and involved it only partially on the anteriomedial side in 3 cases (Fig 48, Table 24)

These macroscopic findings, which established an infarct reaching or failing to reach the papillary muscle, were thus quite strongly correlated ($p < 0.01$) to the observed presence or absence of a mitral systolic murmur

The correlation between the pathologic anatomical and electrocardiographic estimates of the site of a myocardial lesion was good

The size index of recent myocardial wall damage was slightly, but non significantly, higher in the MIMI group, 40 ± 19 cm², the corresponding observation for the no murmur group being 34 ± 22 cm²

The heart weight was usually above normal, the mean being 526 ± 37 g in the MIMI group and 483 ± 35 g in the no murmur group the difference between the groups was significant ($p < 0.01$)

The left ventricular wall had a mean thickness of 17 ± 2 mm in the MIMI group and 16 ± 2 mm in the no murmur group The difference was non significant In 3 MIMI and 3 no murmur cases the right ventricle was thicker than 5 mm

The mitral valve exhibited no pathological changes except for occasionally small degenerative nodules in the valve leaflets No chordal rupture or other damage was detected

The mitral annulus The circumference of the mitral annulus was the same — and within a normal range — in both the mitral incompetence and no-murmur groups In the MIMI group it ranged 95—128 mm with a mean of 110 mm and in the no murmur group 98 128 mm, the mean being 112 mm

Other findings A definite ventricular aneurysm of medium size was detected in two cases involving mitral incompetence and one with no murmur Pericardial affection was observed in 5 MIMI patients and in 4 patients belonging to the no murmur group (total 30 %) Severe mitral annular calcification was present in one case (No 7) Heart rupture occurred in 3 cases in the MIMI group, 2 of them in the posterior upper wall and the 1 in the anterior lower wall Mural trombosis was a common finding despite the prompt initiation of anticoagulant therapy It was found in 53 % of the autopsy examinations Left ventricular mural thrombosis usually apical was present in 53 % of the patients in the MIMI group and in 55 % of the members of the no murmur group In no case were signs detected of the mural thrombus having anatomically prevented closing of the mitral valve leaflets

PATHOLOGY OF PAPILLARY MUSCLES

Patients with the murmur of mitral incompetence developing during the clinical course Appreciable, macroscopically visible necrosis affecting over half the papillary muscle mass was present in 16 of 19 patients (84 %) These changes were noted in 12 cases (75 %) in the posterior, in 8 cases (50 %) in the anterior, and in 4 cases (25 %) in both papillary muscles

Three of the patients had no visible necrosis in the papillary muscles In one case (No 12), the murmur of mitral incompetence developed after electrocardiographic evidence of the progression of a recent inferior infarct to the inferoseptal region had been observed, the patient died of pulmonary edema 6 hours later In another patient (No 6) a macroscopically healthy (but microscopically somewhat necrosed) anterior papillary muscle was attached to the site of a moderate sized aneurysm resulting from a recent anterior infarct (Fig 49)

Table 24 *Degree of the structural destruction of the papillary muscles of the left ventricle in patients who died in hospital*

Case number	Macroscopic necrosis Anterior	Macroscopic necrosis Posterior	Microscopic necrosis Anterior	Microscopic necrosis Posterior	Remarks
	Patients with acute mitral incompetence				
1	−	+	−	+	
2	+	−	+	+	
3	−	+	+	+	
4	+	+	+	+	
5	−	+	+	+	
6	−	−	+	−	Papillary muscle attached to site of aneurysm
7 a	+	−	+	+	
8	−	+	+	+	
9 a	−	+	+	+	
10	−	+	−	−	
11	−	+	+	+	
12	−	−	−	−	Died 6 hours after development of MIMI
13	−	−	(+)	(+)	Transient murmur
14	−	+	+	+	Transient murmur
15	+	−	+	−	
16	+	+	+	+	
17	(+)	+	+	+	
18	+	+	+	+	
19	+	−	+	+	
	Patients without mitral incompetence				
20	−	−	(+)	(+)	
21	−	−	−	−	
22	(+)	−	(+)	(+)	Only tip of papillary muscle necrosed
23	−	−	+	−	
24	−	−	−	−	
25	−	−			
26	(+)	−	(+)	−	Segmental necrosis
27	−	−	(+)	−	
28	−	−	(+)	(+)	
29	(+)	−	(+)	(+)	Segmental necrosis
30	−	−	(+)	(+)	

+ total or subtotal
(+) scattered lesions less than half of cross section areas
− normal finding

The third patient (No 13) had only a transient murmur

No case of total disruption of the papillary muscle appeared One patient (No 17) had a partially ruptured and consequently considerably elongated posterior papillary muscle This change was ascribed to the sudden inception of a loud murmur of grade 4 indicative of mitral incompetence 5 days after the onset of infarction (Fig 50) Death was caused by pulmonary edema resistant to treatment

The heart of one patient with mitral

(Fig 47), and when the infarct was anteriorly situated in the MIMI group the anterior papillary muscle was often significantly damaged In the no-murmur group, although an anterior myocardial infarct might be quite extensive it did not reach the anterior papillary muscle in 8 cases, and involved it only partially on the anteromedial side in 3 cases (Fig 48, Table 24)

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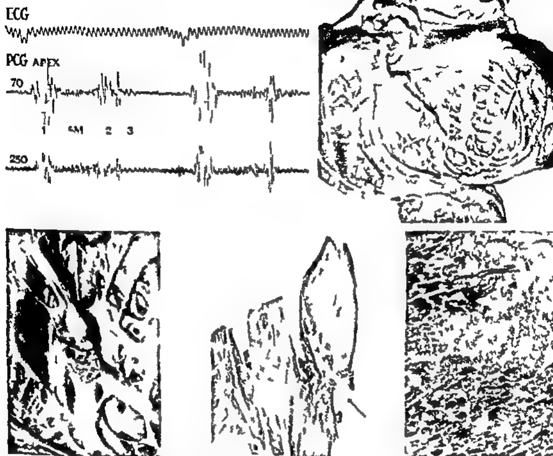


Fig. 50. *Phonocardiogram and autopsy findings from a 58 year old male who developed a harsh grade 4 pansystolic crescendo type murmur (SM) at the apex 5 days after the onset of the infarction. Three days later death occurred from intractable pulmonary edema. An inferoposterolateral infarct (ECG in Fig. 31) involved posterior papillary muscle (P) which was found to be partially ruptured (arrow). Photomicrographs (28 and 58 x) represent evidence of a severe destruction of the posterior papillary muscle. Anterior papillary muscle (A) was also partially damaged by infarct.*

farction. No other consistent differences except location of the infarct were observed in the autopsy findings between patients with and those without mitral incompetence.

The mitral annular circumferences were within normal values in both groups excluding the annular dilatation mechanism of regurgitation.

There was a rather high prevalence of mural thrombosis. It was usually apical, however, and no part of the thrombus was found upon examination in any patient to reach between the mitral valve leaflets and thereby prevent their tight apposition — which was one possibility in a case described by Soulié and Gerbeaux (1939). Further, the relative occurrence of mural thrombosis was about the same in patients without a murmur.




Fig 51 Fibrotic changes in the anterior (A) and posterior (P) papillary muscles resulting from previous infarction The development of MIMI (Fig 50) was followed by a steadily progressing heart failure with markedly enlarging heart volume (Fig 33) Mitral incompetence was confirmed at cardiac catheterization (Fig 58) Circumference of the mitral annulus was 93 mm In addition the anteroseptal region was occupied by a 2 5 cm thick organized mural thrombus (T) Photomicrograph shows an extensive fibrosis of the anterior papillary muscle (3 x van Gieson)

Mitral incompetence was mainly associated with the presence of a posterior infarct The occurrence of an anterior infarct with one exception in all the patients without mitral systolic murmur was remarkable

The critical factor in the development of mitral incompetence in the present investigation seemed to be the presence or absence of significant destruction of one or both papillary muscles by the infarct or distention of its attachment site by an aneurysm forming in the left ventricular wall Burch *et al* 1963)

Another theoretical mechanism could be the loss of active contractility of the mitral orifice (Hurwitt 1953, Davis and Kinmonth 1963) through muscular damage to the base of the ventricle This functional loss of the annular area of the left ventricle can sometimes lead to mitral incompetence without dilatation when it is immobilized by massive calcification (Simon and Liu 1954 Korn *et al* 1962) However in many cases both posterior and anterior infarcts were situated rather low in the ventricular wall Thus, the only pathologic anatomical factor intimately related to mitral incompetence was ischemic papillary muscle damage resulting from infarction

Importance of site and extent of infarct The location of an infarct and its relation to the topographical position of especially the anterior papillary muscle in the left ventricle was of decisive importance in the development of mitral incompetence (Figs 47 and 48)

Posterior or inferior infarct almost invariably involves the posterior papillary muscle (Arkhangelskij 1959) which lies posteriorly near the junction of the free ventricular wall and the interventricular septum

Otherwise the topographical position of the anterior papillary muscle lies on the anterolateral free wall of the left ventricle This position was observed not to be constant but remarkably varied in the present study The anterior papillary muscle does not usually lie at the point opposite to the posterior papillary muscle but more or less laterally In some instances, the anterior papillary muscle was found to have a conspicuously lateral position so that the lines connecting the posterior and anterior papillary muscles to the midpoint of the left ventricular cavity almost formed a right angle (Fig 48)

This marked topographical variation in the position of the anterior papillary muscle could explain the fact that it is possible to have an extensive anterior myocardial infarct destroying nearly half the left ventricular wall circumference but still not involving the anterior papillary muscle if it is situated in a very lateral position (Fig 48) Consequently, no mitral incompetence develops If the anterior papillary muscle was more medially situated it might more easily be involved by an anteroseptal or anterior infarct extending more or less to the free wall of the left ventricle

In posterior infarction this variability of the position of the papillary muscle proved to be only negligible its more medial position almost always ensures that it is involved in posterior infarction

In purely lateral infarcts, the anterior papillary muscle would be more commonly included in the area of the infarct This location of infarct however, seems to be rare (Johnson *et al* 1959)

Although in the MIMI group the size index of myocardial lesions was slightly higher, a small infarct involving the papillary muscle could produce mitral incompetence as easily as an extensive infarct This is comparable to the frequent occurrence of MIMI in patients with subendocardial infarction, as observed in clinical series All the patients had transmural or intramural lesions at autopsy

Degree of papillary muscle destruction The development of mitral incompetence seemed to demand the destruction of a large portion of the papillary muscle In 3 of the 11 patients without murmur of mitral incompetence macroscopic evidence of necrosis in the anterior papillary muscle was observed The area of necrosis was however, segmental and involved only a minor part of the papillary muscle with the main bulk of muscle remaining unaltered (Fig 48)

Two patients in the MIMI group without conspicuous papillary muscle necrosis had only a transient murmur of mitral incompetence which could be explained by borderline ischemic dysfunction of papillary muscle without total or permanent function loss (Burch *et al* 1963) or by acute transient dilatation of the heart pulling down the papillary muscles The end result of papillary muscle destruction by infarction is total fibrosis and function loss of scarred contracted papillary muscle (Fig 51) causing permanent regurgitation (Soulie *et al* 1966 Marton 1966) On the whole mitral incompetence developed at the time when the most severe necrotic myocardial changes had occurred This is not an immediate consequence of a curtailment of the blood supply (Bargmann and Doerr 1963) The same tendency

has been noted in instances of papillary muscle rupture or ventricular septal perforation by an infarct (Chapter II)

Blood supply of papillary muscles The three major coronary artery trunks supplying the papillary muscles have been clearly described (Gross 1921, Spalteholz 1924, Bosco 1935), as have recently the vascular channels at the intramyocardial level (Fulton 1965, James 1965, Estes *et al* 1966) The anterior papillary muscle is supplied chiefly by the left circumflex coronary artery and to some extent by the left anterior descending branch The circulation to the posterior papillary muscle is more variable, depending on the distribution of the „coronary artery predominance" (Schlesinger 1940) Blood to the posterior papillary muscle is mainly supplied by the right coronary artery, as the pattern of right predominance is far more common than the left (Campbell 1929, Schlesinger 1940, Fulton 1965, Lukoff *et al* 1965) In left predominance, the left circumflex coronary artery serves the branches to the posterior papillary muscle This effect of the predominance of coronary circulation explains the frequent involvement of the posterior papillary muscle by occlusion of the right coronary artery

The mode and type of smaller vessels entering the papillary muscles of the left ventricle from the epicardial coronary arteries have been penetratingly revealed by the micro-radiographic technique in the studies of Fulton (1965) and Estes *et al* (1966) The epicardial arteries are arborized perpendicularly to the main vessel and these branches then plunge into the myocardium Two types of smaller intramyocardial vessels can be recognized The branches can rapidly divide into smaller vessels forming a fine meshwork of vessels 400 to 1 500 microns in diameter (Class A vessels of Estes *et al*), after which these divide further within the area of the outer two–thirds of the myocardium Other large branches (Class B) extend in the direction of the ventricular cavity, with only slight forking and without narrowing the lumen These vessels enter the extensive subendocardial network of the large caliber vessels

The papillary muscles derive their circulation from the penetrating arteries directly from the epicardial arterial trunks and the subendocardial plexus Each papillary muscle is supplied by several vessels in a segmental manner Generally the tip, mid portion and base of the papillary muscles receive their own large artery from the epicardial arteries, which also extend branches to the subendocardial plexus Occasionally, the many folds of the trabeculae between the papillary muscle and the myocardial wall carry these vessels to the papillary muscle

In a myocardial infarct, the vascular supply of the papillary muscles is stopped by the obstruction of class B vessels, which results in extensive destruction of papillary muscle Some circulation can take place from the subendocardial plexus of anastomoses (Mitchell and Schwartz 1963, Fulton 1965, Estes *et al* 1966)

In older patients, nonspecific fibrosis of unknown cause is often scattered through the myocardium and the papillary muscles This is in many instances associated with thickening of the small vessels and an anastomotic overgrowth of the class A vessels (Mitchell and Schwartz 1963, Estes *et al* 1966), but the large vessels penetrating to the papillary muscles remain unaltered

The segmental blood supply to papillary muscles could obviously explain the findings of partial destruction of the papillary muscles, as observed in some of the hearts in the no-murmur group Thus, a small, medial, infarcted segmental portion could have been supplied by an occluded anterior descending branch but the main portion was saved structurally and functionally by an uninterrupted main supply of blood from

the left circumflex artery This varying extent of necrosis was not observed in the posterior papillary muscle to the same extent, destruction of the posterior papillary muscle was usually fairly complete The observation that the anterior papillary muscle has a better collateral circulation than the posterior papillary muscle (James 1965) agrees well with the clinical and autopsy findings of the present study

To sum up the findings at autopsy indicated the importance of marked ischemic papillary muscle destruction in the development of mitral incompetence as a consequence of acute myocardial infarction The site of a myocardial infarct in relation to the papillary muscles determines the extent of the damage of muscular structures necessary for competent closure of the mitral valve during systole The more frequent and extensive damage sustained by the posterior papillary muscle is due to the topographical position and the peculiarities of the blood supply to each of the papillary muscles

FOLLOW-UP STUDY

PATIENTS AND EXAMINATION

PATIENTS

An effort was made to arrange follow up examinations for all patients discharged from hospital, in order to observe the permanence and characteristics of the murmur of mitral incompetence as well as the effect of permanent mitral incompetence on the clinical features

The follow up examinations were carried out between 2—11 months after the onset of infarction, the period was generally 6 months For practical reasons it was not possible to fix the same period for the follow up examination of all patients

88 of the 117 patients who had been found to have developed mitral incompetence in connection with myocardial infarction during the period of hospital observation, and 53 of the 70 members of the no-murmur group reported for follow up examination 21 MIMI and 13 no-murmur patients had died in hospital between the time of discharge from hospital and the period of follow up examination the deaths were 5 and 1, respectively In the MIMI group there were 3 patients Nos 7, 7a, 9 9a and III 81a) who were soon rehospitalized because of reinfarction and were included in the patient series These 3 were excluded from the follow up study of first infarctions because only a few weeks ahd elapsed since infarction

EXAMINATION PROCEDURE

The patients attended the outpatients' clinic The same research methods were employed as during the period of hospital observation In recording the case histories, attention was paid to the degree of angina pectoris, congestive heart failure and the use of digitalis In the physical examination, signs of congestive heart failure were looked for, the heart was palpated to reveal any paradoxical pulsation and auscultated for the presence of murmurs and gallop sounds The blood pressure was measured with the patient in a supine position at the final stage of the examination Conventional 12 lead electrocardiogram and phonocardiographic tracings were taken by the author from all the patients after which a chest teleroentgenogram examination was performed by the Radiological Department

RESULTS

INCIDENCE OF PERMANENT MITRAL INCOMPETENCE

In the MIMI group there were 102 patients with permanent mitral incompetence at death or on discharge from hospital 15 patients had had only a transient murmur during hospitalization Of 83 patients with murmur of mitral incompetence on discharge 75 participated in the follow up study (2 having died, 3 not reporting for examination and 3 having been excluded for reasons given in the foregoing)

The murmur was found to persist in 55 patients and to have disappeared in 20 (27 %). Accordingly the murmur was observed to be transient — already disappearing in hospital or within the next few months — in a total of 35 patients out of 117 (30 %).

Of the 70 patients with no mitral systolic murmur (group 2) 53 patients participated in the study. 46 of them were still without a murmur; one had a soft ejection type systolic murmur probably induced by tachycardia on the left sternal border and in the pulmonary region but [illegible] of the patients had developed a systolic murmur indicative of mitral incompetence without having suffered a reinfarction during the period between hospital discharge and the follow up examination.

CHARACTERISTICS OF A PERMANENT MITRAL SYSTOLIC MURMUR

The type of murmur as compared with its prevalent type during the patient's stay in hospital is presented in Table 25. The marked variability in the type of murmur observed in hospital was also evident at the follow up examination. In only 14 instances (25 %) did a permanent murmur remain unchanged in type (out of 55 patients involved) at the time of follow up as compared to 67 (57 % out of 117) during the observation period in hospital.

No crescendo murmur had disappeared although changes to other pansystolic types were common. 20 % of the plateau type of pansystolic murmurs had disappeared and in only [illegible] patients did it remain permanently of the same type, changes to the decrescendo or ejection type being most common. The decrescendo type of pansystolic murmur disappeared relatively most frequently. No late systolic murmur was present and only 1 unchanged early systolic ejection type murmur was observed.

Table 25. *Type of murmur at follow-up*

TYPE OF MURMUR IN HOSPITAL	Pansystolic: Plateau	Pansystolic: Crescendo	Pansystolic: Decrescendo	Pansystolic: Crescendo-decrescendo	Protomesosystolic: Decrescendo	Protomesosystolic: Crescendo-decrescendo	Ejection: Early	Ejection: Late	Total number of murmurs which changed in type	Disappeared	Total
	TYPE OF MURMUR AT FOLLOW UP										
Pansystolic											
Plateau	(8)	4	11		1	1	4		21	12	41
Crescendo	2	(2)	1	1		1			5	[illegible]	
Decrescendo	1	1	(2)		2	1		1	6	7	15
Crescendo-decrescendo			2	(1)			1		3	1	5
Protomesosystolic											
Decrescendo					(0)				0	0	[illegible]
Crescendo-decrescendo						(0)			[illegible]	0	[illegible]
Ejection											
Early		1	1				(1)		[illegible]	2	5
Late										[illegible]	0
Total number of murmur types in o which previous type changed	3	6	15	1	3	3	5	1	3	22	

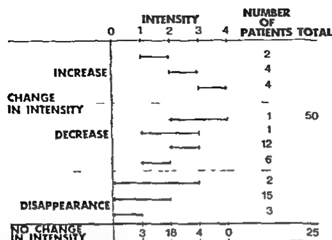


Fig 52 *Changes in the intensity of the mitral systolic murmur on follow-up*

The intensity of the murmur usually decreased as compared to the intensity registered in hospital (Fig 52) In addition to 20 patients with a decrease of intensity to nil, there were 20 patients whose murmur had lessened in intensity In 10 instances the murmur had become louder than during hospital observation, and in 25 (45 %) of the patients the intensity remained unchanged Grade 3 or 4 murmur did not as a rule disappear

Changes in location and frequency The point of maximal intensity of murmur did not vary, in only 2 patients was there a slight shift from the apex to the area between the apex and the left sternal border, and in 1 a shift from this area to the left sternal border was observed

In 10 patients the murmur frequency changed from high to medium in 9, and from medium to high in 1

Relation of the permanence of a murmur at hospital to its presence on follow up It seemed that a constant murmur in hospital did not disappear easily (23 %) as compared to an inconstant murmur (45 %), although not statistically significantly

MITRAL SYSTOLIC MURMURS DEVELOPING IN PATIENTS ORIGINALLY INCLUDED IN THE NO MURMUR GROUP

In [illegible] patients from the no-murmur group who developed mitral incompetence, the murmur was usually apical (5) High frequency was registered in 4 instances, grade 2 in 5, and grade 3 in 1 The murmur was pansystolic in half these patients, 2 exhibiting the crescendo and 1 the decrescendo type, the ejection type occurring in another half

In groups 3 to 5 the murmur was unchanged, apart from minor variations in intensity

OTHER CLINICAL FINDINGS

Angina pectoris occurred more frequently than before myocardial infarction but it did not differ clearly in occurrence or severity in patients with or without mitral systolic murmur Its frequency was estimated to be 73 % in the MIMI group as compared to 81 % in the no murmur group

Reinfarction had occurred in 4 patients with mitral incompetence and in 3 patients with no murmur

Congestive heart failure Subjective symptoms of congestive heart failure were not relatively more common in the MIMI group (41 %) than in the no-murmur group (40 %) Grade IV incapacitating heart failure was much more common however, in patients with mitral incompetence It was observed in 10 patients (28 %) in the MIMI group

but in only 2 patients (10 %) in the no-murmur group In clinical examination 36 % of the MIMI patients were found to have pulmonary rales, as compared to 28 % of the no-murmur group which included the 6 patients with newly developed mitral incompetence among those having rales Signs of congestive heart failure permanently present both during hospitalization and on follow up were observed in 27 % of the MIMI and 9 % of the no-murmur group

12 patients in the MIMI group and 7 in the no-murmur group complained of no symptoms of heart failure, although marked pulmonary moist rales and/or peripheral edema could be detected at the examination Digitalis was used by 49 % of patients in the MIMI group and by 34 % in the no-murmur group There were no noticeable differences between the two groups in the incidence of permanently absent congestive heart failure, the reappearance at the follow up examination of transient failure observed in hospital, and the development of heart failure only during the follow up period

In 37 patients whose mitral incompetence was transient or had disappeared, the signs of congestive heart failure (33 examined at follow up), had vanished with approximately the same incidence as in the patients with a permanent murmur

Paradoxical cardiac pulsation also proved to be a more permanent finding in the MIMI group on follow up 40 patients than in patients without mitral systolic murmur, [illegible] patients The difference was highly significant ($p < 0.001$)

Follow up changes in gallop sounds Third heart sound had about the same incidence on follow up in both the MIMI (28 %) and the no-murmur groups (25 %) The percentages recorded during the ehospital period were 38 and 23 respectively

Fourth heart sound was more closely connected with the acute phase of infarction It was present in only 13 % of the MIMI patients and 11 % of the no-murmur ones on follow-up, while the respective percentages recorded in hospital were 41 and 36

Changes in electrocardiographic findings No consistent differences were observed between the MIMI and no-murmur groups in the degree of ischemic changes in the electrocardiograms Static and limited extension of the ischemic changes as well as their regression or massive extension had the same incidence in both groups

Left ventricular hypertrophy developed or became more marked in 14 patients in the MIMI group, and in only 2 patients in the no-murmur group

[illegible] patients with permanent mitral incompetence developed a P wave duration previously normal of over 0 11 sec, while in the no-murmur group this occurred only in 1 patient The changes in P terminal force and terminal P vector in frontal plane are shown in Figs 27, 28, 29 The marked difference in the P terminal force that developed after MIMI as compared to the patients without mitral systolic murmur persisted during follow up

One patient with mitral incompetence had developed total LBBB Persistent ST segment elevations in the electrocardiogram, pointing to myocardial aneurysm, were observed in 10 MIMI and 7 no-murmur patients

Radiological findings There was a significant ($p < 0.01$) tendency for heart volume to enlarge in the MIMI group (Figs 32 and 33) as compared to the no-murmur patients in the MIMI group 490 ± 102 ml/m² of BSA at hospital and 515 ± 126 ml/m² of BSA at follow up, in the no-murmur group the figures were 478 ± 79 and 488 ± 106 ml/m² of BSA, respectively In patients with transient murmurs this tendency was not observed

No distinct differences were detected in the size of the left ventricle The

frequency of left venticular enlargement increased by 21 % in the MIMI group and by 19 % in the no murmur group On the other hand, the size of the left atrium enlarged more often (23 %) in the MIMI group than in the no murmur group (12 %) Similarly, a demonstrable difference did exist in the grade of the radiological signs of left sided heart failure The pulmonary changes of left sided heart failure (85 %) were usually constant in the no murmur group when the follow up phase was compared to the period of hospital treatment, whereas in the patients with mitral incompetence (79 %) a change to more marked failure was observed The frequency of conspicuous pulmonary venous dilatation increased by 20 % in the MIMI group and by 8 % in the no murmur group The figures with septal lines were 13 % and 8 %, respectively

DISCUSSION

Murmur characteristics The present follow up study further confirmed the great variability of the characteristics of the murmur of mitral incompetence observed during hospital treatment The murmur of acute mitral incompetence after myocardial infarction disappeared in one third of the patients, combining the transient murmurs occurring during the hospital period and the murmurs that disappeared during follow up The constant and loud murmurs disappeared less often than the faint and inconstant ones, which suggests a more marked functional disturbance of the mitral valve in the former

During hospital observation, it was found that the murmur often appeared first as an ejection type before changing to a pansystolic one, especially in the inconstant murmur group In the pure transient murmur group it was often only of the the ejection type (Fig 7) At follow up the ejection or decrescendo pansystolic type of murmur tended to disappear, while part of the plateau, crescendo or crescendo decrescendo types tended to change to an ejection or decrescendo type (25 %)

These findings suggest that the ejection type, early or late, protomesosystolic and pansystolic decrescendo types of murmur associated with mitral incompetence could signify rather minor regurgitation By contrast, the pansystolic plateau, crescendo or crescendo decrescendo types of murmur were observed in connection with both slight and marked incompetence This finding was further supported by the results of the present hemodynamic investigation, it has also been reported by others (Gorlin *et al* 1952, Hultgren and Leo 1959, Nager *et al* 1964, Holldack and Wolf 1966, Leighton *et al* 1966, Ilmurzynska 1966)

The earlier unreported, marked variability of the mitral systolic murmur after myocardial infarction and its often transient occurrence is understandable on the basis of the greatly varied extent of ischemic damage and functional disturbance in the muscular supporting structures of the mitral valve apparatus The state of myocardial contractile power might be an important factor in determining the irreversibility of reversibility of mitral incompetence (see also Chapters VI and XI)

In the no murmur group the development of clinically slight mitral incompetence without infarction was occasionally observed during follow up All the patients, however, had moderate or severe angina pectoris, hence, some slight additional damage to the papillary muscle closure mechanism was possible It is noteworthy in this connection that during the primary study involving "coronary insufficiency" alone, 7 patients developed constant mitral incompetence (p 46) These results show that slight ischemic lesions, often confined to the subendocardial layer, are also capable of causing papillary mitral incompetence

Objective signs of congestive heart failure were more prevalent clinically and radiologically in patients with mitral incompetence, despite the fact that the mitral incompetence was mostly slight in degree judged on clinical grounds

Third heart sound persisted in one quarter of the patients in both groups being a little less frequent than in the acute period Obviously, slight mitral incompetence was not usually its only cause during follow up, the probability is, rather, that the chief cause was cardiac failure (McKusick 1958 Lewis 1962) On the other hand fourth heart sound was detected only in about one tenth of the patients in both groups, although this sound was quite frequently present during the early acute phase It might only be a sign of acute hemodynamic alterations and atrial overloading in the acute phase of infarction as discussed in Chapter VII

The remarkably persistent *paradoxical pulsation* in the MIMI group, as compared to the no-murmur group during follow up cannot be explained solely on the grounds of the presence of congestive heart failure (Harrison 1965) or ischemic bulge (Suh and Eddleman 1959 Davie *et al* 1962, Schweitzer *et al* 1965) but seemed to be connected with the mitral valvular incompetence itself The added total stroke volume of mitral incompetence would probably make the underlying ischemic bulge more easily palpable and recordable, or the heaving of the left atrium could simulate an ischemic paradoxical bulge (Tucker *et al* 1955, Davie *et al* 1962, Logan *et al* 1967)

To sum up the mitral incompetence developing as a result of acute myocardial infarction was obviously of considerably variable severity and often represented a rather unstable phenomenon Thus, the development of permanent severe mitral incompetence as a sequela of of the acute phase of myocardial infarction was not common because of a tendency for the condition to change to intractable, often fatal heart failure At the other extreme the slight incompetence from a minor disturbance of the muscular support of the mitral valve often lessened in severity and frequently completely disappeared This somewhat slight incompetence, however, was not without clinical significance as proved by the frequency of clinical and radiological signs of congestive heart failure

CONCLUDING REMARKS

The aim of the concluding discussion is to sum up briefly and to stress the clinical implications of mitral incompetence developing after acute myocardial infarction. Consideration is also given to the consequences of coronary circulatory alterations affecting the performance of the left ventricle, which are probably related to the development of mitral incompetence. For more detailed comments on the clinical, hemodynamic and pathologic-anatomical findings, the reader is referred to the discussions in the relevant chapters.

CLINICAL IMPLICATIONS

MURMUR CHARACTERISTICS

Incidence. The severe complications causing the appreance of systolic murmur attending a myocardial infarction — perforation of the interventricular septum, rupture of the papillary muscle, rupture of the heart and left venticular aneurysm — are well known but rare, though not exceptional. It is only in recent years that the functional disorder of the mitral valve caused by an ischemic dysfunction of the papillary muscle without rupture has gained clinical attention.

The prevalence of this ischemic functional *impairment of the competence of* the mitral valve seems to be unexpectedly high among patients with acute myocardial infarction. Only two reports (Grotel 1940, Froment *et al.* 1955) give an incidence worth more than a case report. In the present study, the incidence of acute mitral incompetence was observed to be over half the patients with acute myocardial infarction consecutively admitted to hospital. This high incidence is in good agreement with the prevalence of involvement of the papillary muscles observed at autopsy (Arkhangelskij 1959) and with the results of experimental studies (Bailas 1965, Hider *et al.* 1965). The murmur was observed as appearing predominantly during the first five days, when the necrotic changes are maximal (Bargmann and Doerr 1963). In some patients a transient ejection type murmur ascribed to ischemic dysfunction of the papillary muscle developed as early as the first few hours of ischemia.

Intensity. Recognition of this complica*tion demands considerable effort and* careful auscultation. There are two reasons for this: mitral incompetence may sometimes be of very short duration, only hours or days, and a mitral systolic murmur can be, and usually is somewhat faint in intensity. Only rarely, in a third of the patients was it grade 3 or more in the scale of 6. Thus, a murmur can easily be missed in ward examination because of the usual marked background noise

unless it is specifically looked for. Despite its sometimes transient and faint nature, the characteristic pansystolic apical high frequency murmur of mitral incompetence can usually be distinguished without difficulty from the pericardial friction rub when listened to over a period of several days.

It should be emphasized that the faintness of the murmur in no way indicates insignificance. A murmur of only grade 1 or 2 can be caused by hemodynamically severe mitral regurgitation. This clinical impression was confirmed by the hemodynamic portion of the present study and was stressed preliminarily by Gorlin (1966). This is contrary to the general view of the murmur being rather loud (Phillips *et al.* 1963 a, Bashour 1965, Raftery *et al.* 1966), a natural conclusion in retrospective studies. Obviously, a murmur of marked regurgitation can usually be readily detected if it is grade 3 or 4, though there are the important exceptions mentioned in the foregoing. Commonly, the murmur is at first rather faint and only gradually gains in intensity.

Type. Contrary to the reports describing the murmur associated with papillary muscle involvement as being characteristically of an ejection type (Burch *et al.* 1963, Phillips *et al.* 1963 a, Segal and Likoff 1964), the findings in this investigation indicate its character to be typical of the murmur attending mitral incompetence — pansystolic, with a high frequency at the apex. These findings are in agreement with those of other investigators (Grotel 1940, Nezlin and Shamesova 1951, Froment *et al.* 1955, Bashour 1965, Raftery *et al.* 1966).

However, mitral incompetence following myocardial ischemia or infarction seems to be one of the commonest causes of an atypical ejection type murmur of mitral regurgitation located at the left sternal border with occasional radiation to the aortic area (Luisada 1965). This atypical murmur, observed in about 10 % of the patients in the present series as the main type and was the most prevalent as a variant type (28 %), could obviously give rise to difficulties in distinguishing it from murmurs of aortic origin (Schimert *et al.* 1960), if it is not a late systolic variant. Transient occurrence and an eventually frequent change of the ejection type murmur to a pansystolic one at the apex can help recognition of a leaking mitral valve as the cause of this type of murmur.

This frequent later change of an initially ejection type murmur to the pansystolic type, as observed in the present study, though not previously reported, would correspond well to the advancement of an initially only ischemic partial mechanical contraction failure of the papillary muscle (Burch *et al.* 1963), slowly developing in succeeding days to a more extensive necrosis and total loss of function. In the same way as a decrescendo murmur (Nager *et al.* 1964, Holldack and Wolf 1966, Ilmurzynska 1966), the ejection type indicates only a slight regurgitation. This is frequently reversible in ischemic dysfunction of the mitral valve closure and is more common in anterior infarction (Phillips *et al.* 1963 a, Segal and Likoff 1964).

Variability. In addition to the often inconstant occurrence of the murmur, observed in one fourth of the patients, the frequently interchanging types of the mitral systolic murmur in the same patient (43 %) provide further evidence of the dynamic character of the underlying mechanism, structural or only functional ischemic damage to the muscular support of the mitral valve.

CLINICAL SIGNIFICANCE

The clinical significance of the mitral incompetence caused by an infarction of the papillary muscle or of its supporting ventricular wall varies markedly and is obviously related to the amount of the regurgitant flow.

The development of mitral incompetence is not, however, directly related to

of coronary perfusion leading to a regional transmural or subendocardial infarct that includes the papillary muscle Changes in the general coronary circulation, to be considered later, may contribute to this local damage, but only exceptionally do they seem to be the sole cause of mitral incompetence

Accordingly, the topographical position of both papillary muscles in the left ventricle and the site of the infarct chiefly determine the extent of papillary muscle involvement

The association of mitral incompetence with the damage of the posterior papillary muscle (Nezlin and Shamesova 1951, Froment *et al* 1955 Orlando *et al* 1964, Bashour 1965, Raftery *et al* 1966) was confirmed This investigation convincingly demonstrated that the anterior papillary muscle escapes significant infarction more often because of its lateral topographical position in relation to the extent of involvement of the free left ventricular wall by anterior infarction

Distribution of coronary arteries The predominance of the coronary artery distribution seems to have some bearing on the destruction of the posterior papillary muscle In right predominance, present in between 60 and 80 % of the cases (Campbell 1929, Schlesinger 1940 Likoff *et al* 1965 Fulton 1965), the posterior papillary muscle derives its blood supply almost exclusively from the right coronary artery In this case, the posterior infarction resulting from occlusion of the left circumflex coronary artery would not destroy the posterior papillary muscle, which would probably occur in left predominance The scanter collateral circulation to the posterior papillary muscle makes it more vulnerable to extensive ischemic damage than the anterior papillary muscle with its blood supply deriving from both left circumflex and anterior descending coronary arteries (James 1965) These relations might explain the more common occurrence of transient and atypical ejection type murmurs as an indication of slight regurgitation in an anterior as compared to a posterior infarction

Regurgitation mechanisms An ischemic but not necrosed papillary muscle may generate tension in the isovolumetric phase of ventricular contraction, preventing mitral regurgitation during this time, but it fails to contract and shorten during the following ejection phase and thereupon causes regurgitation and ejection type murmur (Burch *et al* 1963) A papillary muscle totally destroyed structurally would allow bulging of the corresponding mitral leaflet into the left atrium immediately at the isovolumetric phase of systole with regurgitation throughout systole This obviously produces pansystolic murmur The same mechanism could also be in operation, causing a pansystolic murmur, when the papillary muscle is attached to the site of an anatomic or dynamic aneurysm, or when an acute general left ventricular dilatation pulls the papillary muscles down (Levy and Edwards 1962, Burch *et al* 1963, Bailas 1965)

HEMODYNAMIC FACTORS

Coronary circulation An acute coronary occlusion leads to profound alterations in the local and general myocardial blood supply and in left ventricular dynamics (Orias 1932, Case *et al* 1954, Wegria *et al* 1954, Gregg and Fisher 1963, Rushmer *et al* 1963, Malmcrona 1964, Bailas 1965, Gunnar *et al* 1966, Mullins *et al* 1966) The determinant of the coronary flow is the coronary driving pressure (Kattus and Gregg 1959, Cross *et al* 1961, Gregg and Fisher 1963) This varies with coronary arterial preussure, coronary vessel vasomotor tonus (which is invariably decreased in ischemic injury) and intramyocardial tissue pressure

Coronary arterial pressure is universally reduced by arterial hypotension In the part of the artery distal to the occlusion it must decrease considerably, resulting in local myocardial damage

Myocardial perfusion Furthermore, general subendocardial ischemia may be induced by the following mechanisms Intramyocardial pressure normally exceeds the intracavitary pressure in the subendocardial layers, decreasing in an epicardial direction (Laszt and Muller 1958, Muller 1962) Thus higher intramural pressure retards the coronary blood flow, especially during systolic contraction Tissue oxygen tension is lower in the subendocardial layers than in the epicardial muscle (Moss 1966) When the left ventricular diastolic pressure is increased over 25 mm Hg and at the same time the coronary arterial pressure is decreased below 70 mm Hg, the intramyocardial pressure overcomes the coronary driving pressure in the entire circumferential subendocardial layer Cessation of coronary circulation in these areas leads to general inner layer ischemia (Salisbury *et al* 1963 a) The contractility of these subendocardial muscle layers including the papillary muscles is disturbed and chordal tension is reduced (Salisbury *et al* 1963 b) The malfunctioning of the papillary muscles as induced by ischemia might thus cause mitral incompetence (Bailas 1965)

Heart dilatation Otherwise, stretching of the myocardium in acute left sided heart failure following a coronary occlusion (Mullins *et al* 1966) is also liable to lead to some impairment of the coronary capillary circulation (Kattus and Gregg 1959) and contribute to myocardial ischemia

Acute left heart dilatation (Mullins *et al* 1966) with or without papillary muscle ischemic dysfunction could bring on mitral incompetence by pulling down the papillary muscles and thus preventing the adequate apposition of the mitral leaflets (Bailas 1965) These two mechanisms, both of them frequently reversible would reasonbly explain the occurrence of initial, often transient, mitral systolic murmurs

This series of hemodynamic alterations could be an important mechanism in perpetuating and promoting a fatal vicious cycle in mitral incompetence following acute ischemic myocardial damage, especially when added to the further inadvertent effect of hypodynamic ventricular contraction in augmenting the regurgitant flow (Wiggers and Feil 1922, Rodbard and Williams 1954, Braunwald *et al* 1957, Gleason and Braunwald 1962, Jose *et al* 1964) — Initial left ventricular failure and shock are common in acute myocardial infarction and connected with hemodynamic mechanisms of general inner layer ischemia experimentally proved in the foregoing However, in the present clinical study they did not appear to provide the main underlying mechanism involved in permanent mitral incompetence, at least This emphazises the importance of papillary muscle damage resulting from local myocardial destruction

It may be stated that the development of mitral incompetence following acute myocardial infarction or ischemia is a frequent clinical manifestation of the pathophysiology of acute coronary heart disease Its highly variable expression is consistent with the underlying cause ischemic heart disease with its remarkably variable structural and functional pathophysiological picture

SUMMARY

Occurrence and clinical characteristics of mitral incompetence developing as a complication of acute myocardial infarction ('MIMI') were investigated in a series of 210 patients with acute myocardial infarction consecutively admitted to hospital. The character and the pathophysiological mechanism of the mitral systolic murmur like the clinical implications and the nature of the hemodynamic disturbance of the mitral incompetence were the main objects of the clinico pathological study.

The patients were observed in thorough daily examinations by the author for the first ten days, and thereafter every other day until discharged. Particular attention was paid to recognizing clinical signs of the congestive heart failure, paradoxical cardiac pulsation and the auscultatory findings of the heart. Murmurs detected were recorded by phonocardiography. Hemodynamic investigations were performed in the convalescent stage on 25 patients with or without acutely developed mitral incompetence, and consisted of right side cardiac catheterization augmented with a pharmacodynamic methoxamine test. Autopsy study of the hearts of 30 patients who died in hospital was made with special reference to the extent of the myocardial infarct and involvement of the papillary muscles of the left ventricle. A follow up after an average of 6 months from the acute phase was carried out to determine the permanence of the mitral incompetence and the clinical progress of the patients.

The main results were as follows.

The prevalence of acute mitral systolic murmur as a complication of myocardial infarction was fairly high, 117 patients developed a new murmur generally within 5 days after the onset of the myocardial infarction. 70 patients had or developed no murmur of mitral incompetence, and in 23 patients the murmur present on admission could not be considered to have been developed recently. Pericardial friction rub was present in 10 % and aortic systolic murmur in 10 % of the patients. The murmur of mitral incompetence was mostly faint, being grade 3 or 4 in the scale of 6 in only one third of patients, thus the murmur can easily escape detection if not meticulously looked for. The faintness of the murmur did not however indicate an invariably insignificant regurgitation, as became evident on the basis of clinical findings and cardiac catheterization data.

The mitral systolic murmur exhibited a remarkably variable characteristics in its occurrence, type, intensity and permanence, even in one and the same patient. Although the murmur of mitral incompetence was overwhelmingly (91 %) apical pansystolic in type, an ejection type as an initial or variation type occurred frequently, with common later transformation to pansystolic. Moreover, if the murmur disappeared later, this happened easily with ejection or decrescendo type murmurs or in pansystolic murmurs first changing to these types. In one third of the patients the acute mitral incompe

tence disappeared in hospital (13 %) or during the subsequent months (17 %) The characteristics of the murmur and the result of the hemodynamic study showed a correlation between the ejection or decrescendo type murmurs and only a slight regurgitation

The variable expression of the murmur is in agreement with its underlying cause ischemic heart disease with its markedly varying pathophysiological picture

The clinical features of this syndrome have a wide spectrum The significantly predisposing factor in the development of mitral incompetence was hypertension and probable predisposing factors were previous infarction and diabetes The clinical, or pathologic anatomical, severity of the infarction was not related directly to the occurrence of mitral incompetence The mitral incompetence was estimated as hemodynamically severe in 17 % of patients Various clinical and radiological signs of left sided heart failure and paradoxical cardiac pulsation occurred very frequently in the patients with acute myocardial infarction, and significantly more commonly in the group with mitral incompetence, often with close time relations In a small portion of patients the acute marked volume load of mitral incompetence superimposed on the acutely damaged left ventricle was obviously an intolerable occurrence, resulting in irreversible pulmonary edema and death

The size of the heart was normal in 55 % of patients with mitral incompetence The left atrium did not dilate acutely The P wave of electrocardiogram showed rapid alterations suggestive of left atrial overloading, most sensitively detected by P terminal force and with significant correlation to the appearance of mitral regurgitation and hemodynamic strain

In the hemodynamic investigation 6 patients had signs of marked mitral regurgitation and severe congestive left ventricular failure whereas 12 patients had a degree of mitral incompetence barely detectable and the hemodynamic alterations were mainly similar to the findings of heart failure observed in 7 patients without mitral incompetence The value of pharmacodynamic pressor amine test in the recognition of slight mitral regurgitation was clearly evident

The critically important determinant in the development of mitral incompetence was convincingly shown to be extensive structural destruction of one or both papillary muscles of the left ventricle Vulnerability of the posterior papillary muscle was apparent The frequent escape of the anterior papillary muscle from major damage was observed to be due to its lateral topographical position in the left ventricle in relation to the site of anterior infarcts All but one 11 patients without mitral incompetence had anterior infarcts without extensive involvement of the papillary muscle in a single case

In 17 of the 19 patients (90 %) with mitral incompetence the papillary muscle predominantly posterior (63 %) was extensively destroyed or attached to the site of ventricular aneurysm, obviously pulling it down during systole with consequent failure of of the mitral valve to close The mechanical failure of the papillary muscle of various modes thus seems to be the cause of the acute mitral incompetence The often observed occurrence of ejection type murmur in the initial phase of infarction might be ascribed to ischemic and only partial mechanical contraction failure of the papillary muscle, the subsequent change to pansystolic type being obviously related to the progress of ischemia to necrosis and total loss of function

However, the functional capacity of the whole left ventricle enters into the mechanism of regurgitation in addition to the papillary muscle damage A fatal vicious cycle may be initiated and maintained by hemodynamic alterations involving waste of contractile speed and strenght of the ischemically injured left ventricle

further enhancing regurgitation Otherwise, the mitral incompetence was frequently unstable and transient The reversibility was probably related to gain of contractile power and diminishing ischemia of the left ventricle, provided that papillary muscle was not severely damaged with the eventual development of scarring fibrosis

Mitral incompetence is a common complication of acute myocardial infarction Its high prevalence and the great variability in its clinical features and severity are not astonishing when considered as an expression of malfunction of the muscular supporting structures of the mitral valve, which are themselves susceptible to ischemic damage of varying degree

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APPENDIX

Abbreviations

Arrhythmia

AF	atrial fibrillation
APB	atrial premature beat
HB 1 2 3	Heart block 1st 2nd or 3rd degree
Nod	nodal rhythm
PB	premature beat
ST	sinus tachycardia
SVT	supraventricular tachycardia
VPB	ventricular premature beat

Phonocardiography

* type of murmur misinterpreted in auscultation
– no difference

Electrocardiography

ICD intraventricular conduction disturbance

CLINICAL ANALYSIS OF PATIENT SERIES

History																	
Case number	Age years	Sex	Angina pectoris	No. of previous infarctions	Cardiovascular disease	Blood pressure mm Hg	Grade of attack	Pulmonary oedema	Heart failure Left Right and acute delayed	Heart failure follow-up	Arrhythmia	Coronary prognostic index	Paradoxical pulsation	Pericardial friction rub	1st sound diminished (D)	2nd sound accentuated (+) or split (S)	
GROUP 1 ACUTE MITRAL INCOMPETENCE PATIENTS EXAMINED AT AUTOPSY																	
1	61	M	−	0		120 −	0		1 Rad		ST	14	+	+			
2	42	M	+	0		90	5	+	Lad		ST VPB	18	+	+		+	
3	30	M	+	[illegible]	Claudication	150 90	0		Ld			7					
4	61	M	+	[illegible]	Hypertension	105/	7	+	LRad		ST AF	24	+	+			
5	40	M	+	[illegible]	Hypert. Rh myocard	100/	7	+	Lad		ST	22	+		[illegible]		
6	57	M	+	1	Hypertension	110 90	0		Lad		VPB	[illegible]	+				
7	[illegible]	F	+	0	Hypert. Fail Hyperchol	110/75	0		Lad		VPB	14	−				
7a	65	F	+	1	Hypert. Fail. Hyperchol	100	5	+	Lad		ST	26	+				
8	60	M	+	2	Hypert Failure	115/	0	+	LRad		ST	20	+				
9	49	M	+	1	Hypert. Diabetes	110 80	5		LRad		HB2 VPB	23	+	+		+ S	
9a	49	M	+	2	Hypert. Diab. Fail	80	7		Lad			21	+			+	
10	[illegible]	M	−	0	Failure	140 80	0		Lad		VPB	14			D		
11	78	M		0		140 90	1		LRa			11	−				
1	73	M	+	0	Hypert. Fail. Perif. art.	180 100	5	+	Lad		SVT	3					
13	47	M	−	0	Hypert. Failure	0 160	0				PB HB1	11	+				
14	31	M	−	0		115/80	0		Lad		HB2	12				+ S	
15	83	M	+	1		95 70	1		1 Rad			13	+				
[illegible]	5	F	+	2	Hypert Diabetes	140 60	7		Lad		HB2	27	+				
17	[illegible]	M	+	0		90 0	7		Lad		VPB	[illegible]	+				
[illegible]	70	M		1		170 5	7	+	LRad		APB VPB	[illegible]	+				
19	39	F		0		110	7		La		HB3 VPB	21	−				
GROUP 2 NO MURMUR, PATIENTS EXAMINED AT AUTOPSY																	
20	34	M	+	0		130/90	0		−		VPB	8	−				
1	6	F		0	Hypert. Failure	160 100	0		Rad		VPB AF	18	+			+	
2?	47	M		0		130 .0	0		La			9	+	+			4
23	60	[illegible]		[illegible]		90 75	7		LRa		PB	1					
24	38	M	+	0	Diabetes	110 80	1		Lad		AF	[illegible]	+	+			
25	64	M		0	Failure Diabetes	115/90	7		LRad		AF	24	+				
26	62	F		0		105/	0		Lad		VPB	14	−				
27	64	M	+	1	Hypertension		5	+	La		VPB f od	25	+				
28	59	M	+	2		130 70	0				PB	11	−				
29	54	M	+	[illegible]		120/90	7					[illegible]	+				
30	64	M	−	0		115 75	1		Lad			11	+	+			
GROUP 1 ACUTE MITRAL INCOMPETENCE																	
[illegible]	57	F		0	Hypertension	150 85	1		Lad	RI	VPB	18	+			+ S	4
3?	4	M	+	0		120 80	0		Ld	−	PB	13					4
[illegible]	51	M		0		135/90	0		Ld	I	PB	8	+		[illegible]		3
34	54	M		[illegible]	Claudication	150 90	0		Lad			7	+			+ S	
35	69	M		0	Hypert. Claudic. Cer vasc.	160/90	0		LRad		AF HB2 APB	[illegible]	+				3
36	58	M		1		130 80	0		Lad	−	HB2	15	−				
37	5?	M		0		125 85	7		Lad	LR	VPB	20	+	+			5
38	61	M		2	Hypert. Fail Diab	160 105	5		Ld		ST	18	−		D		4
39	6?	M		0	Hypert. Cer vasc.	1.0 100	5		Ra	−	Nod AF VPB	25	−				
40	50	M		[illegible]		130 80	5				VPB	13	−				3
[illegible]	60	M	+	0	Hypertension	145 80	0		Lad		AF	14	+		D	+	4
4?	65	M		0	Hypert Diabetes	180/100	0		La			11	+	+			3
43	69	M		0	Hypert. Claudication	155/100	0		Lad		PB	15	+			+ S	3
44	68	F		[illegible]	Hypertension	150 85	0		Lad	L	PB	15	+				4
45	67	F		0	Hypertension	160 90	0		Lad			8	+				
46	39	M		0	Hypertension	1 0 100	5		Lad			6	+		D	+ S	3
47	64	M	+	0	Claudication	150 80	0		Lad			10	+				4

Onset / OA (yrs)	Location (LSB, Ap-LSB)	Transmission	Frequency (H high, M medium)	Permanence (I intermittent, T transient)	Intensity	Type at hospital	Type at follow up	Intensity at follow up	Change at hospital	Change at follow up	COT	Ischemic/transmural	Site	Other findings	Heart volume at hospital	Heart volume at follow up
findings: Mitral systolic murmur												Electrocardiography			Radiology	
4	Apex		H		1		↑				240	T	Inf sept	LAH LVH ICD		
1	Apex	LSB	H		2–3		↑				298	T	Ant	LAH ICD		
OA	Apex		H	I	1→0→2		↑				116	T	Inf	LAH	500	
[illegible]	Apex	LSB	H		2		↑				460	T	ExtAnt	LAH ICD		
3	Apex	Ax LSB	H		1→3		↑				1549	T	Infsept			
6	Apex	LSB	H		1→3		↑				98	T	Antsept Ant	LAH LVH	560	
24	Apex		H	T	2→0						180	T	PostLat	LAH	590	
3	Apex	Axilla	H		2		↑				2300	T	Ant AntLat			
OA	Apex	Ax LSB	H		1→3→4		↑		–		336	T	Inf	LAH ICD		
3	Apex	LSB	H	I	1–0						335	T	InfLat		660	
1	Apex		H		2→3		↑				1330	T	Inf		860	
OA	Apex		M		2–3		↑				8	T	InfPost		810	
2	Apex		H		1		↑				224	T	InfPostLat	LAH		
16	Apex		H		4		↑				42	T	Infsept	LAH LVH ICD		
17	Apex	LSB	H	T	2→3→0		↑				430	T	ExtAnt	LAH LVH	840	
8	LSB		H	T	1→0		↑				[illegible]	T	InfPostLat	LVH		
OA	Apex	LSB	H		1–2		↑				305	T	ExtAnt			
4	Apex		M–H		1→		↑		–		66	T	Inf	LVH	390	
2	Apex	Axilla	H	I	2→0→4		↑		–		270	T	InfPostLat	LAH		
4	Apex	LSB	H		2		↑		–		1450	T	PostLat	LAH ICD		
7	Apex	LSB	M H		1→3		↑		–		2 5	T	ExtAnt	LAH ICD		
											120	T	ExtAnt			
											316	T	Antsept Ant		8.0	
											49	T	Antsept Ant	LVH	500	
											62	T	Antsept	LVH LBBB		
											200	T	ExtAnt			
											80	T	ExtAnt			
											158	T	Ant		450	
											28	T	ExtAnt	LAH ICD		
											28	S	AntLat	LVH		
											260	T	ExtAnt	LAH		
											190	T	ExtAnt	LAH	710	
1	Ap-LSB		H		1 2		–	–	3m	–	270	T	ExtAnt		540	800
OA	Apex	Axilla	H	I	2→0→3		–				188	T	PostInfLat		570	510
7	Apex	Axilla	H		1→3 4			4	–	–	140	T	InfPost		540	730
5	Apex	LSB	M–H	I	1→0–2		–		4m	–	330	T	InfLat		540	510
[illegible]	Apex	Axilla	H		2→3		↑		–		250	T	InfLat	LVH	610	
8	Apex	Axilla	H		2→3			3	–		110	T	Inf		380	350
5	Ap-LSB	LSB	M		1→2 3			3	–	–	? 0	T	InfPostLat		570	5 0
7	Apex	Axilla	H		2				–		68	S	Antsept	LAH	470	
10	Apex	Ax Pulm	H		2			2	4m	4m	840	T	InfLat		540	550
9	Apex	Axilla	H		2→1			2	–	4m	475	T	Inf	LVH	480	510
9	Apex	LSB	H		2			2	–	–	170	T	InfPostLat		480	450
3	Apex		H		1→3			3		–	59	[illegible]	AntLat	LAH	650	6 0
3	Apex	Axilla	H		1→4 3			4	–	–	205	T	InfLat		500	530
5	Apex	Axilla	H		2–3			2	–	–	60	T	Antsept Ant	RBBB	370	5 0
4	Apex	Ax LSB	H		2 3			2	–		18	[illegible]	InfLat		360	460
15	Apex		H		1→2			1		–	95	S	AntLat	LAH	410	410
16	Apex	LSB	M		? 3			2	–	–	[illegible]	T	Antsept Ant		370	380

Table continued

Hs ory																	
Ca e number	Age y ars	Sex	Ang na pec s	No of pre ious arc ons	Ca d o ascu a seases	B ood p es ure mm Hg	G ade of shock	Pulmona y e ma	H e fa e L, R, R da ayed	H art fa u e du ing fo low up	A hy hm a	Coronary p g nd x	Pa adox al pul a on	Pe ard al f on rub	sou d d n hed (D)	2nd sou d en us ed (+) re sp t S	Ga op sounds a hosm al
48	4	F	+	0	Hyperchol	11 80	0		Rad			11	+				
49	55	M		0		90 70	5		Ld		APB	10					–
50	62	M	+	[illegible]	Failu e Clau l	115 60	0				APB	15					4
51	41	M	+	0		110/ 0	1		La	L	ST	10	+			+	
52	4	M		0		105 80	0		La		ST	8		+	D	+	
53	32	M	+	1	C aud a on	1,0 80	0					10	+				4
54	71	M	+	2	Hype e sion	16 90	1		LRad	L		18	+				
55	73	F		[illegible]	C er brovasc	145 100	0		Ld	[illegible]	PB	11	+				
56	49	M		0		1 0 80	0		Lad	LR	ST PB	12	+				3
57	52	M		0		110 65	5		Lad		VPB	17	+			+ 5	3 4
58	66	M	+	0	P nf a	145 00	0			I		6			D		
9	55	M	+	0		140 80	0		La			9	+		D		4
60	45	M	+	0		140 80	0					5					
61	52	F		0		110 80	1		Lad		ST	15	+		D		4
62	58	M	+	0		12 95	0		Ld	L		3					
63	68	F	+	0	Hype ens on	150 100	0		Ld	L		10	+				
64	41	M		0	Hypert ns on	180 115	0					1					
[illegible]	54	M		0	Hype ens on	14 90	0		Ra		PB	8	+				4
66	[illegible]	M		0		110 8	5		LFa		PB HB2 ST	17	+	+			3
67	53	M	+	[illegible]		110 75	0		Lad	L		9	+	+	D	+ S	3 4
68	36	[illegible]		0	Hyper P fa	175 105	0					4				+ 5	
69	61	M		1	Ce vasc P f a	115 80	7		La	L	SVT PB	27	+				4
70	[illegible]	M	+	0		100 7	0		LRa		PB	6					3 4
71	43	M	+	0		120 80	0				PB	5	+				
72	47	M		1	Penf a D abe es	120 90	[illegible]			L	ST	7	+			+ S	3 4
73	43	M		0		1 0 80	0		LRa	L	PB	6	+				
74	49	M		2		20 80	0			L		7	+				
75	66	F		1	Hype Fa u e	160 90	0		La			11	–				
76	53	M	+	1		150 75	0		La	L		1			D	S	
77	49	F		0	Hypert n n	40 80	5		La	L		12	+				
78	33	F	+	0	Hypert Fa D ab	0 0	0		La	LR	PB	1					
79	64	F		0	Diabe es	150 70	0		Ld			3	+				
80	44	M		0		125 85	0		La		VPB	6				+ 5	3 4
81	32	M	+	1	Hype Fa lu	145 90	0					10	+	+		+ 8	4
81a	52	M	+	2	Hyp n Failu	1,0 105	0		La	L		13	+				3 4
[illegible]	56	M	+	1		130 80	[illegible]		Ld	L	PB	16					4
[illegible]	51	M	+	[illegible]	Fa lu e Clau ic	130 90	0		La	L	ST VPB	12	+				3
84	44	M	+	1		1 0 80	0			1	HB1	10			D	S	
85	44	M		[illegible]		130 85	0		LRa	L	VPB	2	+	+	D		3
86	47	M		[illegible]		125/80	0					1			D		4
87	63	F	+		Hyp en io	145 90	0		Lad	1		9					
88	45	M		0		1 5 110	5		Lad		ST	13	+			+ S	3
89	60	F		0		100 70	0		Ra	L	ST	9					4
90	64	M	+	0		14 90	5		Ld			7					
91	61	M		0	Pe f a	149 70	0					5					4
92	55	M		0		2 80	0			L	AF APB	[illegible]	+				4
93	44	M	+	2	Hype ter on	1 0 85	0					4	+			+ 5	3 4
94	34	M		0	Hype ens on	100 70	0		La	L		5	+				4
95	44	F	+			145 90	0		Lad	L	PB	17	+				3
96	40	M	+	0		1 0 75	[illegible]		La			3	+			+ S	
97	61	M		0	Diabe es	120 80	0		La			4				+ S	
98	63	[illegible]		0		150 75	0		Ld		PB	6	+	+			3 4
99	62	M		0	Hype Cla d	19 00	0	+	La	LR	AF VPB	14	+				3
100	41	M	+	[illegible]		40 70	0					5	+				4
101	65	M		0		140 0	0		LRa	L		8					3
102	74	F	+	[illegible]		125 80	0		La	L		9	+			+	
103	61	F		[illegible]		130 75	0		La	LR		7					4

findings												Electrocardiography			Radiology	
	Mitral systolic murmur															
On ... OA ...	Location LSB ... Ap-LSB	T...	Frequency H high M medium	I inconstant T transient	Intensity	Type at first	Type at follow up	Intensity at follow up	at hospital	at follow up	COT max	1st ... T transmural	Site of infarction	Other findings	Heart volume at hospital	at follow up
8	Apex	LSB	H		1→?			2	4m	—	47	T	Ant		350	410
12	Apex		H		3						260	T	InfPostLat			
2	Apex	LSB	M		1→?–3			3	—	—	165	T	InfPost		4 0	480
1	Apex	LSB	M	I	2→0→?			3	3 4m	—	280	T	ExtAnt		470	8 0
8	Apex		H		2→3			?	—	—	34	T	PostInfLat		690	670
8	Apex		M		1→2			2	3m	4m	188	T	Inf	LBBB	590	530
2	Apex		H		1–2			2		—	29	T	InfLat	part LBBB LVH	430	490
9	Apex	LSB	M–H	I	1→0–2			2		—	23	S	Antsept		360	410
8	Apex	Axilla	H		2→1→3			2		—	180	T	Inf		560	630
1	Apex	Pulm	H	I	2→0→2		—	—	—	—	2?5	T	Antsept Ant		420	380
3	Apex	Ax Ao	H	I	3→0→2			2	—	—	256	T	InfLat	LAH	490	440
4	Apex	Ax Ao	M–H		3→			3	—	—	97	T	ExtAnt	LAH	570	570
4	Apex		H	I	1–0→2						30	T	Antsept Ant		440	
2	Apex	LSB	H	I	1→2			2	—	—	31	T	Ant		490	640
9	Apex		H		1			2		—	50	T	PostLat		490	450
5	Apex	LSB	H	I	2→0→?		—	—	4m	—	31	T	Infsept		560	600
25	Apex		H		1→2			1		—	46	S	InfLat		450	410
5	Ap-LSB		H		2–3			3		—	270	T	Infsept	LAH	590	570
28	Apex		H		2–3				4m		300	T	InfPost		490	470
26	Apex	LSB	H		2			0–1	—	—	30?	T	ExtAnt	LAH RBBB	340	350
2	Apex		H		2						33	T	PostInfLat	LVH	470	
4	Apex	Axilla	H	I	2–0–1			2		—	303	T	PostInfLat	ICD	550	630
4	Apex		H	T	2→0				—		242	T	ExtAnt		530	
5	LSB		H	T	1→0		—	–		—	273	T	Antsept Ant		430	440
4	Apex		H	T	1–2→0		—			—	6?	S	InfLat		440	680
3	Apex	LSB	H	T	1–2→0		—	—	4m	—	200	T	InfPostLat	LAH	500	500
3	Apex		H		2		—	—		—	26	S	Antsept Ant	LAH	430	450
1	Apex		H		1→0→		—		—	—	60	S	Antsept Ant		370	390
1	Apex		H	I	2→1→3		—	–			350	T	InfLat		510	490
4	Apex		H		2		—	–	4m	—	20	S	Antsept Ant	LAH	410	400
1	Apex		H	I	1–0→2			2	—	—	33	T	Inf		500	380
7	Ap-LSB		H		?→1		—			—	28	S	ExtAnt		430	440
11	Apex		H	T	1–0→?		—	—		—	150	S	AntLat		430	450
10	Apex		H	T	1→0						120	T	InfLat	LAH		
1	Apex		H	T	1–2→0		—	—			54	T	Ant	LAH	550	630
1	Apex	LSB	M	I	2–0		—	—	—	—	186	T	InfPost	LAH	420	450
14	Apex		H	T	1–2→0		—	—		—	156	T	AntLat	LAH	430	480
1	Apex		M–H		1→2			2	3 4m		69	S	Infsept	LVH	460	590
5	Apex	Axill	H		3			2	4m	—	464	T	PostLat	LAH	550	570
5	Apex	Axilla	H		1→2→3			2	—		60	S	Antsept Ant		410	380
OA	Ap-LSB		H	I	2→0→2		—	—		—	52	S	Ant AntLat		400	410
3	Ap-LSB		H		1→2 3		—	—	—	—	86	T	Ant	LAH	550	610
5	Apex		H	T	2→0		—	—	—	—	?0	T	Antsept Ant		470	440
3	Apex		M		2						2	T	InSept AntLat			
4	Apex		H		1 2			1	—		37	S	InfPost		430	460
3	Apex	LSB	M		2–3			2	—	—	120	T	Ant AntLat	LAH	460	600
1	Apex	Axilla	H	I	2→0→3			2	—	—	80	S	AntLat		410	3 0
1	Apex		M–H	I	2→0→2			1	—	—	163	T	ExtAnt	LAH	4 0	470
5	Apex	Axilla	H		?→3–4			4	—	—	220	T	InfLat	LAH LVH	670	760
3	Apex	LSB	H		1→3			2	—		98	T	Antsept Ant		490	4 0
4	Apex		M–H	I	2–0		—	—	—	—	154	S	PostLat	LAH	510	530
4	Apex		M		1→2			—	—	—	173	T	InfLat		580	550
5	Apex	Axilla	H		1→1→3			4	—	—	96	S	ExtAnt	LVH	650	730
13	Apex		H		2→1			2	—	—	288	T	Antsept Ant	LAH LVH	400	400
5	Apex	Ax LSB	H		2–3			2	—		54	S	Ant-AntLat		400	410
2	Apex		H		2–3			1		—	50	T	Ant		330	380
1	Apex		H		1→2–3		—	—	—	—	127	T	Antsept Ant		390	420

Table continued

History																		
Case number	Age years	Sex	Angina pectoris	No of previous infarctions	Cardiovascular ...	Blood pressure mmHg	Cardiac shock	Pulmonary edema	Heart failure L R delayed	Heart failure at follow up	Arrhythmia	Coronary prognostic index	Paradoxical pulsation	Precordial friction rub	1st sound diminished (D)	2nd sound ... (+) or ... (S)	Gallop sound ...	C
104	64	M	+	6	Failure Claudice	120 80	0		Lad		PB	20	+	+	D		3	
105	5	M	+	1	Claudication	150 90	0		La	LR	VPB	12	+			+	3 4	
106	44	M	+	0	Claudication	125/90	7	+	La		ST VPB	21	+			+ S	3 4	
10	60	F	+	3	Pe f a t	135 85	0			LR		[illegible]						
108	61	F		0	Failure Perif a t	135 75	[illegible]		Lad	LR	AP	14	+			+ S	3	
109	33	M		0	Hype pidemia	1.0 90	0				ST	3	+				3	
110	42	F		0		115 55	0					7	+					
111	72	M	+	1	Hypertension	135/100	0				PB	14	+				4	
112	5	M	+	1	Claudication	140 85	0			L	AF VPB	14						
113	.8	M	+	0		140 [illegible]	0					[illegible]				+ S		
114	43	M		0		130 40	0		LRa		ST	[illegible]		+			4	
115	60	M	+	3		120 90	0					9	+		D			
116	54	M	+	1		160 90	0		Ld	L	ST APB	13	+		D	+ S	3 4	
117	4.	F	+	[illegible]	Hype t Failure	1 0 80	1	+	La		ST	18	+		D	+		
118	62	M		1	Hype t. Ce asc	140 90	0			LR		12	+				3	
119	54	F		0		105 65	0		Lad	LR	VPB	14	+		[illegible]	+		
120	[illegible]	M	+	0	Failure Claud	180 85	1	+	Lad	L	VPB	16						
121	1	M		2		110 65	3		Lad	L	HB3	21	+		D		3	
122	48	M		0		140 8	0			R		4						
1 3	50	M	+	1	Failure Ce vasc	130 90	5					15					3 4	
124	38	F	+	0		115 85	3		La	L	HB3	20						
125	55	M	+	0		110 70	7	+	La	L	HB3	21						
GROUP 2 NO MURMUR																		
126	[illegible]	M		0	Failure	163 90	0	+	La	L		11					4	
127	35	M		0		150 85	0					9						
128	61	M	+	0		130 70	0				PB	6					4	
1 9	65	M		0		140 95	0		La			8					4	
130	54	M	+	1	Claudication	130 90	0				ST VPB	14						
131	64	M	+	0	Claudication	135 90	[illegible]				VPB	6						
132	56	M		0		110 70	[illegible]		Ld	L		5				+	4	
133	38	M		0	Hype Fa l Ce vasc	230 130	0		La	L	ST	15					3	
134	76	M		0	Hypertension	180 10	0		LRad	1	HB1 VPB	15					4	
135	73	F		0		140 80	0	+	Lad	I	AF	13						
136	47	M	+	2	Failure Pe f a	110/80	1		Lad		VPB	15					4	
137	47	M		0		135 95	0		La	I	ST	1					3	:
138	60	M		0	Claudication	140 80	0			L		[illegible]						
139	40	M		[illegible]		110 70	5					1						
140	56	M		[illegible]		105 70	0					5						
141	9	M		[illegible]		130 90	5		La		SVT HB2	17	+				3 4	:
142	53	M		0		12 175	0				PB	5						
143	62	M	+	0		120 70	3		La	L		16				+	4	
144	78	M		0		1 0 85	0		La	L	ST PB							:
145	33	M		0	Go	145 100	0					4	+					
146	57	M		0		105 70	5		Ld			15						
147	53	M		0		130 90	0		La			5	+				4	
148	53	M	+	[illegible]		80 60	1		La			7						:
149	64	M		1		130 8	0			I		5						
150	69	M	+	[illegible]	Failure	150 85	7			L	VPB	17						
151	39	M		0		145 100	0					4	+			+	4	
15?	49	M		[illegible]	Rh myocarditis	110/70	0		La			8					4	(
153	47	M	+	[illegible]		130 0	0			[illegible]		2	+				4	
154	[illegible]	F		[illegible]	Hypertension	170/105	0		Ra	L		11						
155	65	F	+	0		150/100	0			I		5						
156	53	M	+	[illegible]	Hyper Claudic	125 80	[illegible]		Lad	I		11	+				4	
157	69	M		[illegible]		155	[illegible]		La	I		11						

Findings												Electrocardiography			Pathology	
Mitral systolic murmur																
OA	Location (LSB; Ap-LSB)	Transmission	Frequency H high M medium	Permanence I inconstant T transient	Intensity	Type at hospital	Type at follow-up	Intensity at follow-up	Comparison to phonocardiography: at hospital	at follow-up	QT max	Extent	Site of infarction	Other	at [illegible] Heart weight	at [illegible]
1	Apex		H		1–2–3				4m		46	T	ExtAnt	LVH ICD	670	
2	Apex		M	I	2–0			2	–		43	S	Ant	LAH	520	400
9	Apex		[illegible]	I	1–0–2						400	T	F tAnt	LAH	580	
3	Apex	LSB	H		1–2			–		–	[illegible]	S	Ant	LVH	431	420
OA	Apex		H	I	2–0			3	–	–	112	T	ExtAnt		570	600
4	Apex	LSB	H		2–3			2	–	–	36	T	InfLat		410	410
4	Apex		H		2			–	–	–	25	S	A tsept Ant	LBBB	380	[illegible]
2	Apex	LSB	M		2–1–3			1	–	–	42	S	Ant ept Ant	LAH LVH	550	340
3	Apex	Aortic	H		2			–		3m	34	S	I f	LAH	500	[illegible]
2	Apex		H		1			–	–	–	100	T	PostInfLat		530	570
4	Apex		H	T	1→3→0			–		4m	320	T	InfLat	LAH	610	460
1	Apex	LSB	M–H		2→3			2	–	–	132	S	Ant-AntLat		[illegible]	480
3	Apex	Axilla	H	I	3→0→4			2	–	–	130	T	AntLat	LAH	540	370
1	Apex	LSB	H	I	3→0→3			2	–	–	430	T	F tAnt		530	610
1	Apex	LSB	M–H		2			3	–	–	240	T	ExtAnt	LAH	[illegible]	380
4	Apex		H		1			1	–	–	104	T	Antsept Ant	LAH	[illegible]	370
2	Apex	LSB	H	I	1→0→2			2	–	–	165	S	ExtAnt	LAH LVH	[illegible]	710
5	Apex	Axilla	H		1→4–3			3	–	–	900	T	Antsept-Ant	LAH LPBB	1080	1130
2	LSB	Pulm	M	T	2→0					–	162	T	PostLat		[illegible]	460
[illegible]	Apex		M	I	1→0→2			–		–	58	T	Antsept-Ant	LVH	[illegible]	500
25	Ap-LSB		M		3			2	–	–	160	T	F tAnt		370	440
9	Ap-LSB	Apex	M–H	T	2→0			–	–	–	2 0	T	L tAnt	LAH RBBB	[illegible]	[illegible]
								2		–	31	T	PostInf		480	720
								2		–	30	S	ExtAnt		410	[illegible]
										–	33	S	Antsept	LAH	480	470
								2		–	64	T	A tsept A t		[illegible]	[illegible]
										–	35	T	Antsept-Ant		510	520
										–	100	T	InfPostLat		570	[illegible]
										–	131	T	Antsept-Ant	LAH	540	[illegible]
										–	112	S	Ant	LAH LVH	[illegible]	700
										–	63	S	Antsept-Ant		500	[illegible]
										–	68	S	Antsept-Ant		480	[illegible]
										–	132	T	Ant	LAH ICD	680	[illegible]
										–	58	S	Antsept-Ant		440	410
										–	39	S	InfLat	LAH	420	[illegible]
										–	[illegible]	T	InfPostLat		[illegible]	[illegible]
										–	[illegible]	T	Antsept-Ant		500	[illegible]
										4m	328	T	Antsept-Ant	LAH	520	500
										–	73	T	AntLat		410	[illegible]
										–	124	T	Ant-AntLat		[illegible]	570
										–	77	T	AntLat	LAH PBPP	[illegible]	[illegible]
								2		–	40	S	Antsept-Ant		340	440
										–	[illegible]	T	Ant	LAH	norm	
										–	120	T	Post	LVH	610	410
										–	232	T	ExtAnt	LAH	[illegible]	560
										–	[illegible]	T	PostInfLat		[illegible]	400
										–	80	T	Antsept-Ant		[illegible]	[illegible]
										–	115	T	Antsept-Ant	LAH	510	
										–	225	T	Antsept-Ant		[illegible]	440
										–	33	S	Ant-AntLat		320	[illegible]
										–	40	T	Antsept		320	290
										–	[illegible]	S	Ant-AntLat		[illegible]	510
										–		T	Antsept-Ant		610	[illegible]
										–	119	T	Inf		[illegible]	440

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 476

CURRENT TRENDS IN DIABETES MELLITUS

In Honour Of

NIELS B. KRARUP

Dedicated Papers
On The Occasion Of His Sixtieth Birthday
July 14 1967

COPENHAGEN 1967

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CURRENT TRENDS IN DIABETES MELLITUS



ACTA MEDICA SCANDINAVICA

Supplementum No 476

CURRENT TRENDS IN DIABETES MELLITUS

In Honour Of

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On The Occasion Of His Sixtieth Birthday
July 14, 1967

Copenhagen 1967



EDITORIAL

The present collection of original studies is dedicated to N B Krarup on the occasion of his sixtieth birthday

The volume aims at providing a survey of present scientific activity in diabetes research in Denmark The editorial committee has therefore collected contributions from a number of investigators actively engaged in this field of research Some of these contributors have not collaborated directly with N B Krarup in a professional capacity, and the editorial committee is particularly grateful to them for their participation As a result of their contributions, it has been possible to widen the scope of this volume

The special nature of the subject has necessarily limited the circle of contributors and excluded scientific contributions from a number of N B Krarup's former and present pupils These and other colleagues who wished to contribute to the publication of this dedicatory volume in some other manner have been listed in the *tabula gratulatoria*

The printing of this volume has been made possible by
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NIELS KRARUP

Niels Bygom Krarup, on his seventieth birthday, July the [illegible], is an impressive personality within the field of Danish internal medicine, well-known among his colleagues in the medical world and in wide circles outside.

Internal medicine of the last forty years has been dominated by its increasing progress and new approaches to diagnostic and therapeutic problems. During these years Niels Krarup has become a leading figure among Danish internists due to his vast knowledge, memory, his clinical intuition and sharpness of observation. The open and [illegible] mind of Niels Krarup, his interest in human behaviour, his broad experience and ability for [illegible] and just decisions inevitably made him an appreciated adviser not only to his colleagues but also to many other groups depending on medical knowledge.

In his daily life Niels Krarup is head of Medical Department C in the new section of our old Bispebjerg Hospital, one of the municipal hospitals of Copenhagen. At the same time he is an associate professor, a teacher in clinical medicine for the medical students. These are his two principal activities within a broad spectrum of medical and non-medical interests, not only within science but also covering many other aspects of our culture and often in giving a direct appeal to the public in the current debate on the problems of our society.

Niels Krarup was born to a life in medicine. His father, Jens Kristian Birch Krarup, M.D., was a highly qualified pediatrician and at the same time a physician and practitioner of the good old days, a man who yielded an enormous amount of work and for whom it was part of his life that, with ever-lasting care and sense of duty, he should be at his patients' disposal day and night.

This sense of duty came naturally to Niels Krarup in his own education and training as a physician and as a defender of the individual patient. Niels Krarup's mother was a Heerfordt and related to the well-known ophthalmologist.

In 1932 Niels Krarup graduated and entered the medical profession. The need and demand for medical research in these years was great with even greater expectations of therapeutic results. It was a happy coincidence, therefore, when Niels Krarup was given the opportunity of working with H. C. Hagedorn at Nordisk Insulin Laboratorium in the practical development of protamine insulin and its administration in the treatment of diabetes mellitus.

Niels Krarup's theoretical studies and practical clinical activity at Niels Steensens Hospital developed into a life-long and profound interest in the pro-

INTRODUCTION

Knud Lundbæk

Diabetes mellitus as a subject for scientific investigation dates from the middle of the 19th century It was inaugurated by the brilliant studies of Claude Bernard in carbohydrate physiology, the numerous clinico-chemical investigations of ketoacidosis by Kussmaul, Gerhardt and others, and by an ever increasing flow of clinical studies of the natural history of diabetes mellitus, including, already at the time, observations on diabetic vascular disease by Marchal de Calvi and the first descriptions of diabetic retinopathy, which appeared shortly after Helmholtz s invention of the opthalmoscope

It is evident that from the very beginning the study of diabetes mellitus was established as an area of research uniting in collaboration and discussion investigators from many disciplines, clinicians as well as non-clinicians

It also appears that three of the major subdivisions of diabetes research were already under discussion a hundred years ago the physiology of the diabetic state, the metabolic derangement in diabetic patients, and diabetic angiopathy The fourth was introduced in 1889 by Mering and Minkowski's pancreatectomy diabetes, opening the way which today has led to the study of diabetes mellitus as an endocrine disease

The greatest event in the history of diabetes research was, of course, the discovery of insulin by Banting and Best in 1922 In 1935 Hagedorn produced the first long acting insulin preparation, the clinical effects of which were studied in detail by N B Krarup

The last 30 years have witnessed an immense expansion in all fields of diabetes research High lights of immediate practical importance include the zinc insulins, sulfonylurea and biguanides, as well as a much better understanding of how to handle the problems of diabetes and pregnancy Our knowledge of the course and prognosis of diabetes mellitus has been increased, especially regarding the development of the sinister vascular abnormalities which determine the fate of today's diabetic patient Mass investigations of whole populations have revealed surprising new facts in the field of diabetes epidemiology, and the difficult problems of the inheritance of the disease are coming to the fore An understanding of the minute details of the changes in intermediary metabolism in diabetes has grown concurrently with the tremendous growth in biochemical research in general and its application to the study of human diabetes, as well as to the study of spontaneous diabetes in animals and the many varieties of ex

perimental diabetes The introduction of assay methods for blood hormones, especially the immunological techniques, has now made it possible to consider the pathogenesis of the diabetic state as an abnormality of the secretion or action of insulin and other hormones on various tissues

The contributions of Danish diabetologists to this volume *in honorem* N B Krarup reflect the extensiveness and the many sidedness of diabetes research They range all the way from classical clinical studies to experimental clinical, physiological and biochemical investigations, the authors trying to answer or at least to elucidate a number of pertinent questions

What is the mode of inheritance in diabetes mellitus? Are juvenile and late-onset diabetes genetically different conditions? (Harvald) Should diabetes be included in the fashionable group of autoimmune diseases i e are there reasons to believe that diabetes or long term diabetic manifestations are due to the formation of auto-antibodies against insulin? (Deckert) What is plasma insulin? Are there several kinds of insulin circulating in the blood, with different biological and physiological characteristics? (Lyngsoe) What happens to insulin when it is labelled with radioactive iodine for use as a tool in biochemical or clinical research? (Brunfeldt) What is the physiological significance of the action of insulin on the central nervous system? (Rafaelsen) How should we interpret the increase in fatty tissue blood flow during acute intravenous injection of concentrated glucose solutions in contrast to the fall observed after slow infusion? (Quaade et al)

Important therapeutic problems are reviewed What is the balance of wanted and unwanted effects of the insulin preparations available today? (Poulsen) Why is it that blood sugar rises to a higher level after breakfast than after meals at other times of the day? (Jersild) How does sulfonylurea work to produce hypoglycemia? (Joop Madsen)

Diabetic angiopathy is discussed from several points of view What is the evidence for a demonstrable functional abnormality of the capillaries early in the course of diabetes mellitus? (Ditzel) What is the significance of the increased capillary diffusion capacity for sodium ions shown to be present in long term diabetes? (Trap-Jensen et al) And what is the role of abnormal coagulability in the development of diabetic angiopathy? (Valdorf-Hansen) What can we learn about diabetic angiopathy from observations on vascular changes in long term experimental diabetes in animals? (Lundbæk et al)

Finally what are, and what were in an earlier period, the principle causes of perinatal death in diabetic pregnancy? (Molsted Pedersen and J Pedersen)

It is hoped that this collection of essays will be of interest to diabetologists as well as to research workers in related fields of medicine and basic disciplines It also affords a picture of some of the subjects—not all—that occupy the mind of some—not all—of the Danish investigators working in the field of diabetes mellitus today

From the Department of Medicine C (Heads N B Krarup M D and B Harvald M D)
Bispebjerg hospital Copenhagen Denmark

GENETIC PERSPECTIVES IN DIABETES MELLITUS

by

Bent Harvald

Diabetes mellitus presents some of the most intriguing problems in clinical genetics In spite of the abundance of accumulated data it has not yet been possible to establish the mode of inheritance with any degree of certainty All attempts at fitting in diabetes with any known Mendelian system seem to imply the disregard of important evidence Among the impediments to a proper genetic analysis, the most serious is ignorance with regard to the nature of the basic defect Moreover, it is unknown whether the defect is the same in all cases so heterogeneity cannot be excluded

The incidence of diabetes in the general population is not well defined, especially with regard to the older age classes where borderline cases are numerous and the diagnosis not very clear-cut The broad period of manifestation from early childhood to old age and the different ages of onset in the two sexes necessitate adjustments These factors complicate the testing of any genetic hypothesis drawing on the concept of gene frequencies in the general population and the expected frequencies in different groups of relatives

Importance of genetic factors, twin studies

Observations of single pairs of monovular twins concordant with regard to diabetes, where the disease runs nearly identical courses in both twins, have been frequently reported ever since the turn of the century, leaving the impression of a high concordance rate in monovular twins This has been confirmed in several twin series, collected more or less systematically (5, 49, 42, 43, 39, 20, 17)

In 1938 Then Berg (40) published the first series of diabetic twins collected in accordance with the principles laid down by Luxenburger (22) for unbiassed sampling in twin studies Out of 49 monozygous (MZ) pairs 19 showed clinical concordance, in 10 of the discordant pairs glucose tolerance tests were performed in the clinically unaffected partners, 13 of whom were classified as having an abnormal curve of diabetic type, whereas 6 were normal In 11 discordant pairs the test could not be applied because the unaffected partners had died by the time of the investigation The corresponding figures for 86

dizygous (DZ) pairs were as follows 9 pairs were clinically concordant, 11 were concordant after glucose tolerance test and 35 were discordant Glucose tolerance tests could not be applied in 31 cases

The difference between MZ and DZ pairs is highly significant Certainly the clinical concordance in MZ twins is far from unity, but it must be taken into consideration that the unaffected partners have been observed only through a limited span of years after the onset of diabetes in the proband

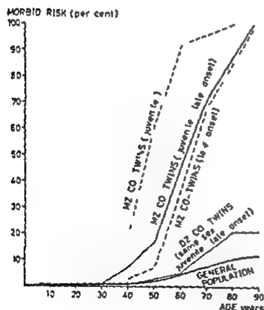


Fig 1

Morbid risk of co-twins of diabetic twin probands calculated according to Nyholm & Helweg Larsen (27) Juvenile in this figure indicates diabetes in the twin proband diagnosed before the age of 50 years The risk for the general population has been calculated by Degnbol ( ) on the basis of Fitzgerald et al s series (11)

In a population of about 8,000 pairs of twins born in Denmark (14, 15) in the years 1870–1910 and followed up from birth and all through their life, 97 cases of diabetes have been diagnosed in MZ twins, 154 cases in DZ same-sexed twins and 139 cases in DZ different sexed pairs At the time of investigation 58 of the 97 MZ co twins were known to have diabetes, contrary to 14 out of 154 co-twins in the DZ same-sexed pairs and 12 out of 139 co-twins in the DZ different-sexed pairs As the age distribution in the three groups of twins is very similar, the concordance rates can be directly compared, showing a highly significant difference between MZ and DZ same-sexed pairs

In the discordant pairs, 7 unaffected MZ co-twins and 11 unaffected DZ co-twins had their fasting blood sugar repeatedly examined, and in cases where it was normal or borderline, glucose tolerance tests were performed (32) 4 of the 7 MZ co-twins and 1 of the 11 DZ co twins showed a clearly diabetic pattern

As diabetes must be expected to manifest itself later in some of the unaffected co-twins, it was found of value to estimate the morbid risk of the co twins by a life table procedure (27) It appears from fig 1 that the morbid risk approaches 100 per cent in MZ co-twins, but the standard deviation is considerable In the DZ co-twins the morbid risk in all age classes is significantly lower, reaching a level at about 20 per cent In early-onset diabetes the MZ co-twins develop the disease at an earlier age than the MZ co-twins of late-onset diabetics

These different twin studies justify the conclusion that the appearance or non appearance of diabetes is totally or nearly totally determined by genes, and the environmental factors are more or less confined to influencing the time of on set and probably also the course of the disease

Family studies, recessive inheritance

The well recognized familial concentration of cases of diabetes mellitus has been placed on a more sound statistical basis through several systematic family studies (3, 1, 28, 21, 13, 38, 41, 18, 12, 19, 26, 33, 34, 7, 6) Diverse simple genetic mechanisms have been suggested, but most authors assign a simple Mendelian recessive mode of inheritance to the predisposition for diabetes (36, 37, 38)

Under the assumption that all diabetes is due to one and the same recessive gene, the gene frequency in Western populations should be expected to be very high, thus the morbid risk in an English population (10) has been estimated to be over 10 per cent at the age of 80 years, which means a gene frequency above 30 Calculated on this basis the morbid risk in sibs of diabetics is over 40 per cent, in the offspring it is 30 per cent at the age of 80 years One tenth of these cases will appear before the age of 40 Thus, with this very high gene frequency in the general population the disease expectancies in sibs and offspring do not differ very much

A comparison between age specific morbid risks for sibs and offspring in three existing materials is given in table 1

The figures do not directly contradict the hypothesis of recessivity as the standard deviations are rather large, especially those of the risk figures for offspring On the other hand, a simple recessivity theory cannot without difficulty explain the apparent heterogeneity Thus the morbid risk both for sibs and offspring of late-onset diabetics does not differ significantly from that of the general population before the age of 40, where

TABLE 1

Comparison between morbid risk of diabetes in sibs and offspring

	Sibs	Offspring
Morbid risk at the age of 15 years Probands with juvenile diabetes mellitus (Degnbol 1965)	3 70±0 98 %	2 78±1 66 %
Morbid risk at the age of 40 years Probands with severe diabetes mellitus nearly all juvenile (Grunnet 1957)	5 70±1 28 %	9 46±3 47 %
Morbid risk at the age of 40 years Probands with late onset diabetes mellitus (Harris 1950)	0 58±0 04 %*)	1 38±0 39 %*)

*) Calculation by Degnbol 1965

it is only about one tenth of the morbid risk for sibs and offspring of juvenile diabetics. This difference between relatives of juvenile and late-onset diabetics is even more clearly demonstrated by the risk curves (fig. 2). For both types of diabetics the curves for offspring and sibs seem to lie very near to each other.

A critical test for the recessivity theory is afforded by the findings in the offspring of conjugal diabetics. If all cases are due to recessive inheritance involving the same locus, all the offspring of two affected parents should themselves be affected. The following crude figures have been reported:

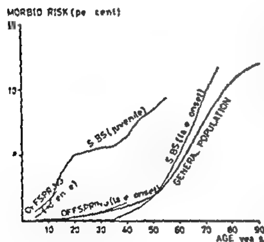


Fig. 2

Morbid risk of relatives of diabetics compared with that of the *general population*. Offspring of juvenile diabetics (Degnbol's series (6)). Sibs of juvenile diabetics (Degnbol's series (6)). Offspring of late-onset diabetics (Harris' series (13), calculation by Degnbol (6)). Sibs of late-onset diabetics (Harris' series (13), calculation by Degnbol (6)). General population (Fitzgerald et al.'s series (11), calculation by Degnbol (6)).

Pincus & White 1934 (29) 30/137
Steiner 1936 (39) 9/30
West and Post 1962 (48,30) 13/161
Simpson 1964 (34) 5/173
Cooke et al. 1966 (4) 16/362

These figures immediately seem to be incompatible with the hypothesis of simple recessive inheritance. Analysis with due regard to age composition confirms this impression (34, 4).

Neel & al. (25) have analysed the offspring of different marriage types: diabetes × diabetes, diabetes × normal, normal × normal, with application of glucose and cortisone glucose tolerance tests (10). Highly significant aberrations of the mean tolerance curve from that of the normal controls could be demonstrated in offspring of conjugal diabetics and offspring of diabetes × normal marriages. In this latter group simple recessivity would involve a bimodal distribution of the test results which, however, could not be demonstrated. On the assumption that an abnormal glucose tolerance test or cortisone glucose tolerance test is an indication of prediabetes, more than 50 per cent of the offspring of the diabetes × normal crosses could be considered as affected. This is a clear excess over expectation if the disease should be due to homozygosity for a recessive gene occurring at a single locus, unless the frequency of the gene in the population is as high as 50. Especially in youths, however, the value of the glucose tolerance test or the cortisone glucose tolerance test in detecting prediabetic conditions is questionable, and some authors have failed to demonstrate any difference between young unaffected

near relatives of diabetics and normal controls (23, 26)

Increased consanguinity in parents of juvenile diabetics has been reported by Harris (13), but the comparability of his series with the control series used (consanguinity in hospital inmates, studied by Bell (2)) is questionable Von Kries (18) in an extensive series of diabetics was not able to confirm Harris' results Steinberg & Wilder (38) found a higher consanguinity between parents of late onset diabetics than between parents of juvenile diabetics The interpretation of the figures is not easy, however Under the assumption of a frequency of the diabetes gene of 10 and with about 0 5 per cent marriages between first cousins in the general population, only about 0 8 per cent of all recessive homozygotes in this population should be expected to be derived from first cousin marriages Thus it will be very difficult to register with certainty a difference of consanguinity between parents of diabetics and the general population Furthermore the population pattern changes from one generation to the next and from one area to the other

The recessivity theory has to some extent been supported by observations in animals with spontaneous diabetes In the house mouse a type of diabetes combined with obesity has been described with a clearly recessive mode of inheritance (16) Diabetes in the Chinese hamster (24, 50) seems to be much more similar to human diabetes It was originally thought that diabetes in the Chinese hamster was also due to a single recessive gene, but this has not been confirmed by later breeding experiments and the somewhat complicated genetic situation agrees rather with multifactorial inheritance Thus diabetes in hamsters represents all degrees of severity The infertility of the severely affected animals, however, is an obstacle to genetic experimentation

Insulin isolated from the serum of untreated juvenile diabetics has been found more resistant to destruction by a crude rat muscle insulinase than insulin from normal controls, the insulin of parents of juvenile diabetics shows an insulinase resistance in between (31) These results indicate some structural change in the diabetic insulin molecule The series published so far, however, are limited and the findings need further confirmation

Combined recessivity and dominance

The morbid risk in sibs and offspring of late-onset diabetics does not differ significantly from that of the general population before the age of 40, contrary to the morbid risk in sibs and offspring of juvenile diabetics, which in the younger age groups is 5–10 times that of the general population (cf fig 2) This point was first stressed by Harris (13) and has been verified in later materials (12, 6) It led Harris to the conclusion that diabetes is genetically heterogenous As the correlation of age of onset within sibships is high, but low between parents and children Harris advanced the hypothesis that patients with mild late-onset diabetes are heterozygous for the same gene, which in homozygous form gives rise to a severe early onset diabetes This theory is very attractive and fits in well with many empirical ratios

1) With a gene frequency in the general population of 05–10, predisposition for the mild type of diabetes should be expected in 10–20 per cent of the population—in accordance with the observed morbid risk of 12 per cent at the age of 80

2) The parents of juvenile diabetics should be expected to be either heterozygotes or homozygotes whereas only one parent of late-onset diabetics must necessarily carry the gene Thus the number of clinically affected parents of juvenile diabetics should be expected to be about twice as high as that of parents of late-onset diabetics The empirical figures from Steinberg & Wilder's material (38) calculated by Degnbol (5) are

Risk at the age of 50 of parents of juvenile diabetics 1 62 per cent,

Risk at the age of 50 of parents of late-onset diabetics 0 88 per cent

3 Among the offspring of juvenile diabetics 5–10 per cent should be expected to develop early-onset diabetes, which is in agreement with observation

In other respects, however, Harris hypothesis does not explain observed data Thus about 30 per cent of sibs of juvenile diabetics should be expected to be similarly affected, which is three times the observed number 3–6 per cent of sibs of late-onset diabetics should be expected to develop juvenile diabetes This is more than three times the observed figure The morbid risks for sibs till the age of 40 are rather well established figures in both groups Thus the discrepancy between expectancy and observation makes it necessary to discard this hypothesis in spite of its merit of simplicity

Irregular dominant inheritance

Irregular dominance of one single gene was proposed as a theoretic possibility first by Levit & Pessikova (21), later supported by v Kries (18) The ratios observed in different groups of relatives with approximately the same percentage of affected sibs and offspring are consistent with this hypothesis, presupposing an extreme phenotypical variability and low penetrance The varying expressivity of the gene could be partly an effect of environment, partly of modifying genes Modifying genes and common environment could explain the age correlation within sibships

A precise test of the dominance theory is not possible at present The hypothesis would be strengthened if some sort of bimodal distribution with regard to one or other trait could be demonstrated among the sibs and offspring of diabetics The study of the distribution of glucose tolerance by Neel & al (25) failed to demonstrate such bimodality

The dominance theory has been strongly supported by the studies of Vallance-Owen and his collaborators (44, 45, 46, 47) who have demonstrated the existence of an insulin antagonistic system associated with plasma albumin Excessive antagonism of this type has been found in patients with juvenile as well as late-onset diabetes Moreover, clinically unaffected relatives of diabetics have a high plasma 'synalbumin' antagonism Thus, about half the sibs

and half the offspring have been found to be "synalbumin positive", suggesting inheritance as a dominant trait Only a minor part of the "synalbumin positive relatives had an overt carbohydrate in tolerance

These findings have been confirmed by Erlich & al (8), who among 27 sibs of diabetic children found 11 with excessive antagonism The biochemical character of the synalbumin antagonist is not definitely established, but some observations indicate that the antagonist is identical with the B-chain of the insulin molecule (9)

The data published so far do not allow a strict genetic analysis Insulin antagonism is a quantitative trait, thus in 5 per cent concentration the plasma albumin from all subjects has an antagonistic effect The limit between normal and excessive antagonism is an arbitrary one Therefore the absolute numbers of relatives with excessive antagonism must be estimated with reserve A multifactorial trait may feign monomerism if the arbitrary limit between normal and abnormal is adequately placed The demonstration of a bimodal distribution of the actual quantitative estimate of insulin antagonism would in this respect be of crucial importance

Among 28 patients with coronary occlusion Vallance Owen and Ashton (47) found excessive insulin antagonism in 19, compared with 6 out of 28 controls of the same age These findings indicate that high insulin antagonism is a very common finding, at least in the older age groups

The causal relationship between excessive insulin antagonism and diabetes has not been definitely established To decide whether insulin antagonism is a suitable biochemical marker to ascertain the diabetic genotype, long term observations will be necessary Thus it is of interest to find out to which extent clinically unaffected individuals with excessive insulin antagonism will develop diabetes later on

Multifactorial inheritance

In their study of glucose tolerance in offspring of diabetics, Neel & al (25) found a distribution not differing significantly from unimodality On this basis they suggested a hypothesis of complex multigenic inheritance involving additive effect of genes at several different loci Multigenic inheritance would readily explain several data

1) High concordance in MZ twins (approaching 100 per cent) combined with a very much lower concordance in DZ twins (about 20 per cent)

2) Morbid risks for sibs and offspring of approximately the same size

3) Higher morbid risk for sibs if one or both parents are also diabetic Thus Steinberg & Wilder (38) found the following incidences for sibs

Both parents unaffected	4 7 per cent
One parent diabetic	11 4 per cent
Both parents diabetic	16 0 per cent

It should be stressed that these figures are not risk figures, and they have not been corrected for age

A simple multifactorial system, however, does not account for the low risk of juvenile diabetes in sibs and offspring of late-onset diabetics. This age specificity would presuppose a more complex system with additional genes determining the age of onset.

The distribution of secondary cases of diabetes in different groups of relatives suggests that the mode of inheritance may be different in juvenile and late onset diabetes. In Grunnet's series (12) 11 probands with severe early-onset diabetes had more than one affected relative in the ascending line. In 10 of *these families the secondary cases were* either on the maternal or the paternal side, only in one were family members of both sides affected. 7 probands with mild late-onset diabetes had more than one affected relative in the ascending line. In 5 of these families the secondary cases were found on both maternal and paternal side. This difference is statistically significant ($P < 0.01$). The late-onset diabetes is the one which tallies best with a multifactorial pattern.

The diabetic genotype

Convincing evidence has been accumulated to the point that non diabetic relatives of diabetics differ from the average population in many respects. A comprehensive anthropological study by Nilsson (26) elucidates this problem. Relatives of diabetics were found to be taller and heavier than the controls, with increased skeletal sturdiness. The same was the case in young diabetics examined before the onset of diabetes. Female diabetics over the age of 50 had given birth to more children than non-diabetic women. Children of mothers who developed diabetes afterwards, had a birth weight on an average 400 g over normal. This may partly be ascribed to the higher weight of these mothers and partly to the higher average parity number, but there are some suggestions that the high birth weight of these children of prediabetic mothers may at least to some extent be due to the diabetic genotype. Thus, children of prediabetic fathers and children who later on developed diabetes themselves also had an average birth weight significantly over normal.

The assessment of the phenotypical characteristics of the diabetic genotype is difficult, however, so long as a reliable biochemical marker is not available. The study of unaffected MZ co-twins of diabetics is theoretically a sound approach, but the high concordance in MZ twins impedes the collection of a sufficient sample for conclusive analysis.

COMMENTS

The present status leaves an impression of confusion. Without any doubt genetic factors play the major role in the aetiology of diabetes, juvenile as well as late-onset. There is much evidence that these two types of diabetes are genetically different. Late-onset diabetes in many respects behaves as a multifactorial trait due to additive effect of genes at several loci, thus it presents all degrees of severity, the less severe cases by far outnumbering the severe ones. Irregular dominance, however, cannot be excluded, in which case several modifying genes must be active. From a clinical point of view juvenile diabetes is much

more similar to a monomeric disease entity The onset of the disease is rather abrupt and the majority of cases after a short period of time reach a level where insulin activity of plasma approaches zero and all insulin must be supplied from without In this respect juvenile diabetes resembles an inborn error of metabolism due to a single enzymatic defect Formal genetic analysis, as well as the biochemical demonstration of a plasma synalbumin antagonist, favour dominance, but neither recessivity nor multifactorial inheritance can be totally ruled out

Several points need further elucidation The determination of plasma insulin activity and insulin antagonism in clinically unaffected partners of MZ twin pairs discordant with respect to diabetes will be of crucial importance in the evaluation of excessive insulin antagonism as a biochemical marker of the diabetic geno-type More family studies of this trait are necessary for the performance of a strict genetic analysis, including longitudinal studies of non-diabetic relatives with excessive synalbumin antagonism, in order to establish the constancy and significance of this trait in the single individual Studies more or less on the same lines are desirable with regard to insulinase resistance of insulin in diabetics and their relatives

In future clinico-genetic studies the heterogeneity problem should be considered carefully The morbid risk figures for several groups of relatives have been indicated only with a high degree of uncertainty because of insufficient data Thus, follow-up studies are needed of 1) offspring of juvenile diabetics followed up to middle and old age, 2) sibs of juvenile diabetics followed up to old age, 3) offspring of late-onset diabetics followed up to old age Moreover, the existing series of 4) offspring of conjugal juvenile diabetics are of limited size and do not allow calculation of good risk figures for this very important group

Reliable morbid risk figures for different ethnic groups would be of major importance for the elucidation of the heterogeneity problem It is well known that the incidence of diabetes differs much from one population to another This is true both in juvenile and in late-onset diabetes It would now be of interest to know whether the incidence of juvenile and of late-onset diabetes in a given population are always lowered or increased more or less to the same degree If this is not the case, i e if for instance a high incidence of late-onset or mild diabetes can be found together with a low incidence of juvenile and severe diabetes, this would give strong support to the heterogeneity hypothesis

Family studies of clinical subgroups might also be informative, for example genetic studies of early-onset diabetics with low insulin requirement

Studies of this type, however, may not result in any significant clarification It is more probable that conclusive progress on the genetic front must await basic pathophysiological advances

SUMMARY

The elaboration of the genetic theory in diabetes must take some well-established data into consideration

1) The concordance in MZ twins approaches unity both in juvenile and late onset diabetes. The concordance in MZ twins is significantly higher than that of DZ same sexed twins.

2) The morbid risk of offspring and sibs of juvenile diabetics is nearly of the same order of magnitude, about 3 per cent till the age of 15, 5–10 per cent till the age of 40.

3) The morbid risks of offspring and sibs of late-onset diabetics are of nearly the same order of magnitude and do not differ significantly from that of the general population until after the age of 40.

Juvenile and late-onset diabetes are probably genetically different, but this question as well as the question of the mode s of inheritance cannot be solved until a reliable biochemical marker of the diabetic genotype has been found. The synalbumin insulin antagonist system is perhaps an approach in this direction.

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AUTOIMMUNOLOGICAL ASPECTS OF DIABETES MELLITUS

by

Torsten Deckert

The molecular structure of the polypeptide, insulin, is well known, and insulin has been successfully synthesized An analysis of the amino acid sequence has shown that the primary structure of the insulin molecule presents only small differences from one animal species to another (fig 1 and 2) In spite of this, however, the injection of insulin from one animal species into another animal species can give rise to the formation of antibodies (14) Insulin antibodies can also be demonstrated in man after treatment with pig or ox insulin for 1–2

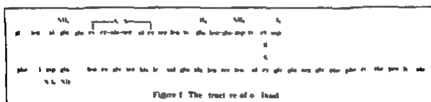


Fig I

Primary structure of ox insulin

Figure 2.

Spe i s-spe ifi ity of i sulin.

The table show the ami o acid h racteristic f r pecies- pecifi ity situated t A8 A9 A10 a d B30.

	A8 – A10		A10	B30
i sulin	1	ser	i	al
sei whal i sul	al	ser	th	ala
heep i suli	al	gly	i	ala
horse i sul	thr –	gly	ileu	ala
perm-whal sulin	thr	se	leu	al
pig i suli	[illegible]	ser	ileu	al
rabbit i suli	th	ser	ileu	ser
h m n insuli	th	ser	ileu	thr

Fig II

Differences in primary structure of insulin from different animals

months The demonstration of insulin antibodies in diabetics has raised the question whether the pathogenesis of diabetes may be conditional on the presence of insulin antibodies, and whether some of the complications of long standing diabetes are the result of immunological reactions between insulin and insulin antibodies, whether, in short, diabetes mellitus may be regarded as an autoimmune disease

For diabetes mellitus to be considered as an autoimmune disease, it is necessary that the diabetic organism should respond with immunological reactions to contact with its own species specific components, and that these immunological reactions should lead to tissue damage

Various mechanisms may be imagined which might bring about autoimmune reactions 1) a modification of the molecular structure of species specific components 2) absence of tolerance, and 3) an alteration in the function of the immunological apparatus

Changes in the molecular structure of the insulin molecule

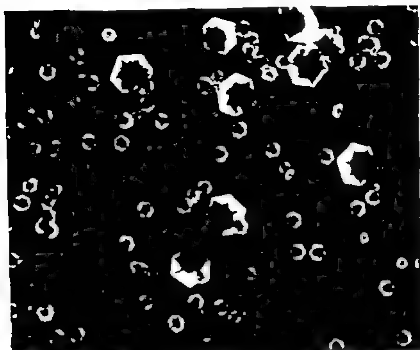
Insulin in particular has of course been in the limelight among those species-specific components which might become heterologous as a result of changes in the molecular structure

Very little is known of the biosynthesis of insulin in the beta-cells of islet tissue (47) and it is uncertain whether the synthesis of insulin and/or the insulin release mechanism is abnormal in diabetics It is well known, however that the majority of diabetics who acquire the disease after the age of 40 years have an abnormal glucose tolerance, in spite of a high serum insulin concentration (5) The explanation of this phenomenon could be that maturity-onset diabetics secrete a biologically inferior insulin

There was no difference, however, in the appearance of crystals of insulin extracted from the pancreas of elderly deceased diabetics and deceased non-diabetics, respectively (fig 3) Nor could any difference be demonstrated immunologically, and the preparations showed an identical ability to lower the blood sugar level in rabbits (8) (fig 4 and 5) A comparison of the immunological reactivity of serum insulin in diabetics and non diabetics likewise showed no differences (15)

To date, therefore, it has not been possible to demonstrate any structural differences between insulin from non-diabetics and insulin from diabetics Modifications of insulin are known, however, which deviate so strongly from the normal structure of insulin that they only react very slightly with the insulin antibodies against normal crystalline ox, pig or human insulin Examples of this are guinea-pig insulin (25, 34) and fully iodinated insulin (4, 7)

With the techniques employed in immunological studies, it will be difficult to reveal serum insulin modifications with a pronounced degree of deviation In spite of the investigations mentioned above, therefore, the possibility cannot be excluded that in diabetics, small amounts of insulin are produced which from an immunological point of view have a pronounced degree of deviation However, with the methods which are



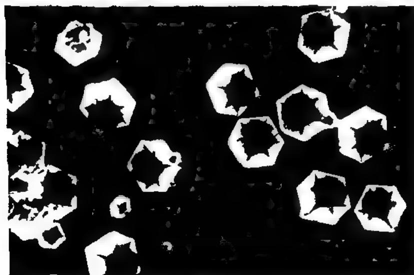


Fig III

Photomicrograph of insulin crystal suspension
above human insulin from non-diabetics
below human insulin from stable diabetics
(from Brunfeldt et al 1966)

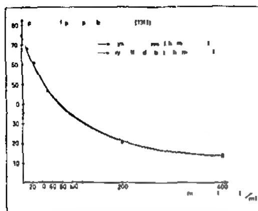


Fig 11

Inhibition of the reaction between pig insulin antibody and ¹³¹I pig insulin with
— crystalline insulin from non diabetics
x—x crystalline insulin from stable diabetics
(from Brunfeldt et al 1966)

employed for studying insulin antibodies, it should be possible to reveal antibodies against such an insulin preparation, provided it possessed at least a minimum immunological relationship with crystalline insulin As it has not been possible to reveal such antibodies (see later), the possibility of such an insulin being produced must be regarded as minimal Furthermore, considering the smallness of the insulin molecule, it must be regarded as unlikely that the beta cells in diabetics synthesize an "insulin" which differs so much from normal insulin that a cross reaction with normal insulin anti bodies cannot occur at all

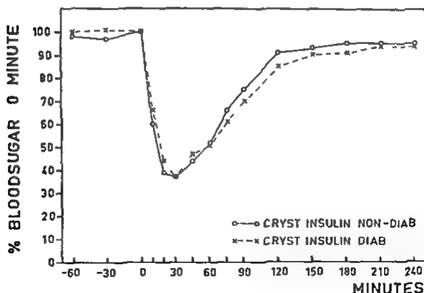


Fig 1

Hypoglycaemic activity of
o—o crystalline insulin from non-diabetics
x—x crystalline insulin from stable diabetics
20 μg crystalline insulin/kg was injected into the marginal ear vein of 8 rabbits in cross-over experiments (from Brunfeldt et al 1966)

Isoantibodies

As no immunological differences could be demonstrated either between insulin from diabetics and non-diabetics or between pancreatic insulin and serum insulin (15), it is striking that isoimmunization with insulin has repeatedly proved possible It has proved possible to immunize both pigs cows sheep and rats with species specific insulin (6, 27, 31, 44 50), and preliminary studies suggest that this is also the case in man (16) It was considered that the use of adjuvant (14) or protamine (31) would have the effect of modifying the insulin molecule and thus making it antigenic (4) However, it has also been possible to immunize pigs by means of daily injections of an acid solution of crystalline pig insulin (6, 50), i e without the use of adjuvant (fig 6) On the other hand it has not been possible so far to isoimmunize animals by using neutral solutions of highly purified insulin without adjuvant (50) The possibility cannot be excluded therefore, that the inflammatory tissue reaction elicited by daily in



Fig 11

Immunoelectrophoresis and autoradiography of pig serum to which approximately III µg [131]I pig insulin has been added per ml serum

A autoradiography of serum before treatment with insulin

B autoradiography of serum after treatment with pig insulin

The arrow indicates [131]I insulin bound to gamma globulin (from Brunfeldt & Deckert 1964)

jections of unphysiological fluids (43) may be of significance for the response of the organism to the injected insulin Likewise, the unphysiologically high concentration of antigen at the site of injection may be of significance (14, 31, 44) Finally, it should be mentioned that most commercial preparations of insulin contain strongly antigenic impurities, which can potentiate the formation of antibody by insulin (14)

Humoral insulin antibodies

Just as it has proved impossible to demonstrate changes in the insulin molecule in diabetics, so it has proved impossible to demonstrate with certainty the presence of insulin antibodies in diabetics not treated with insulin If the pathogenesis of diabetes depended on an alteration in the molecular structure of insulin, with resulting formation of antibodies, insulin antibodies would be expected to be found In non insulin-treated diabetics, the complement-consumption test has actually been found positive in 58 per cent of the cases, but it was also positive in 26 per cent of non diabetics (11) The experimental results were interpreted as expressing complement-binding to a serum protein insulin complex It should also be mentioned that in three diabetics not treated with insulin, gamma globulins were demonstrated which when labelled with fluorescein isothiocyanate and dripped on to fresh human pancreatic tissue resulted in fluorescence of beta cells (33) However, it [redacted] possible that the phenomenon is nonspecific

All other investigations undertaken with radioactive isotope labelled human or pig insulin as antigen have given negative results (14) (table 1)

There is thus no evidence for the presence of humoral antibodies in diabetics prior to the commencement of insulin treatment It should once more be mentioned, however, that the investigations undertaken are only able to reveal antibodies which correspond with the normally occurring antigenic determinants of pig, ox or human insulin It will only be possible to reveal the formation of antibody directed against newly

TABLE 1

Results of experiments with ^{131}I insulin for insulin antibody detection in different groups of diabetics and non diabetics

Number of patients investigated	Group	Insulin treatment	Labelled insulin used as antigen	Insulin antibodies found in serum (percentage)
26	juvenile diabetics	none	pig	0
11	elderly diabetics	none	pig	0
45	non-diabetics	none	pig	[redacted]
6	juvenile and elderly diabetics	none	human	[redacted]
[redacted]	non-diabetics	none	human	0
5	diabetics	all	human	100
93	diabetics	all	pig	[redacted]

formed determinants in the insulin molecule provided there is an antigenic relationship between these determinants and the determinants of the genuine antigen.

Cellular insulin antibodies

Even though it has not been possible to demonstrate humoral insulin antibodies in diabetics not treated with insulin, the possibility of cell bound antibodies being present cannot be excluded. Federlin et al. (23) demonstrated the presence of lymphocytes sensitized against insulin in patients with insulin allergy. In some cases, these lymphocyte bound antibodies were found before humoral insulin antibodies could be demonstrated. This is in agreement with the observation that insulin allergy directed towards highly purified insulin may be found in patients who do not have humoral insulin antibodies (14). In diabetics not treated with insulin, however, no sensitized lymphocytes could be demonstrated. It should also be emphasized that the erythematous, warm, indolent, slightly elevated injection infiltrations, about 2.5 to 3 cm in size, which occur in some diabetics and which must be regarded as a delayed hypersensitivity reaction of the tuberculin type, are never seen immediately after the commencement of insulin treatment, but only after the lapse of some days, i.e. some weeks before the humoral insulin antibodies can usually first be demonstrated.

Insulitis

As stated, autoantibodies and/or immunologically abnormal insulin could not be demonstrated in diabetics. Certain pathological lesions in the pancreas, however, localized in and around the islets in animals and man, have kept the discussion open on the question of the autoimmunological genesis of diabetes. There are three different findings. 1) infiltration of eosinophil leucocytes, particularly peripheral to but also in the hypertrophic islet tissue of the pancreas in infants born to diabetic mothers, 2) infiltration of round cells and polymorphonuclear leucocytes in and around the islet tissue in juvenile diabetics who died in the course of 6 months after the disease was diagnosed, and 3) infiltration of round cells and/or polymorphonuclear leucocytes and/or eosinophil leucocytes in the pancreas of animals which have been actively or passively immunized with insulin.

Re 1) infants born to diabetic mothers and dying during the perinatal period, show infiltration of eosinophil leucocytes around hypertrophic islet tissue. This lesion has been familiar for a long time, and its specificity must now be considered established. The reason for the changes, however, is still unclear. There seems to be no relationship between the occurrence of humoral insulin antibodies and the development of eosinophil infiltrates around the islet tissue, as even considerable infiltrates can be seen in infants whose diabetic mothers have never been treated with insulin (46), and in whom insulin antibodies therefore usually do not arise. It is possible that these lesions should be regarded as a reaction to an islet mass which is proliferating strongly on account of hyper-

glycaemia They can hardly be regarded as harmful to function, as it has been shown that infants born to diabetic mothers produce considerably more insulin following intravenous injection of glucose than do normal infants (26) (fig 7)

Re 2) Inflammatory pathological lesions in the islet tissue in persons who have died with a relatively recently diagnosed diabetes have previously been observed only rarely (35, 41) After the autoimmune hypothesis was put forward, however, interest naturally increased considerably (40, 29) Thus, Gepts (24) states having seen leucocyte infiltration in 15 out of 22 cases of juvenile diabetes with a duration of less than 6 months The lesions always appear to be accompanied by changes in the cells of the islet tissue, such as shrinkage and cell degeneration (41) The manifestation is independent of the administration of insulin (37) It is likewise noted (29, 37) that lymphocyte infiltration is not found in and around *all* the islets of Langerhans in an affected pancreas This argues against the presence of immunological processes The possibility cannot be rejected that the lymphocyte infiltration is a reaction to a cell degeneration of particularly rapid onset Neither humoral nor cellular antibodies in the islet tissue could be demonstrated in diabetics not treated with insulin Thus, there are no good grounds for assuming that the pathological changes described as insulitis should be based on immunological reactions

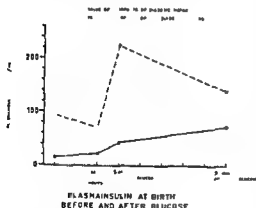


Fig 7 II

Plasma insulin in infants of non diabetics and of diabetic mothers at birth (cord blood) and during the first few hours of life in the fasting state before and after a rapid glucose injection through an umbilical vein catheter (umbilical vein blood) (from Jorgensen et al 1965)

Re 3) Pathological lesions have been described repeatedly in and around the islet tissue in animals who have either been actively or passively immunized with insulin (28, 32, 44, 48, 49, 50) In some animals, these changes appear as a massive round cell infiltration, in other cases as an infiltration of eosinophil cells in and around the islet tissue, and in still other animals as a diffuse infiltration of polymorphonuclear leucocytes in the pancreas It is probable that these reactions are a direct or indirect sequel to an antigen antibody reaction, whereas they are hardly an expression of a toxic effect of the antigen antibody complex (28) It should be mentioned, however, that the majority of commercially available insulin preparations contain antigenic impurities, which do not have an antigenic relationship with insulin, but originate from the pancreas and therefore can confuse the picture to a high degree, provided the insulin preparation used for the experiments is not highly purified (14) Nor can the

possibility be excluded that the lesions are non specific and determined by the sudden considerable strain on the production of insulin, with cell death as a sequel This is supported by experiments which show that pathological changes in islet tissue with round cell infiltration only occur in rabbits which had become diabetic during the active immunization with insulin, while immunized rabbits without diabetes had normal pancreas histology (48) In partly pancreatectomized animals, however, made diabetic by injection of pituitary extract, no round cell infiltration was observed in the islet tissue, in spite of a considerable drain on the insulin production (18)

It is difficult to make comparisons between the various pathological lesions described as insulitis, since they originate from different individuals and animal species There are both peri insular infiltrations of eosinophil leucocytes, intra-insular round cell infiltrations or infiltration of polymorphonuclear leucocytes No evidence can be brought forward to refute the conclusion that a common feature of the lesions is that they are a sequel of antigen antibody reactions in and around the islet tissue Further studies must be made with respect to demonstration of bound insulin antibodies in the pancreas especially in man, before the question can be settled

Manifestations of long-standing diabetes

It was suggested by Berson et al (3) already in 1956 that certain manifestations of long-standing diabetes might be due to immunological reactions conditional on the presence of insulin antibodies Since then, however, it has been possible to establish that there [redacted] no relationship between the demonstration of humoral insulin antibodies and the manifestations of long term diabetes (14) It has now also been confirmed that both nodular glomerulosclerosis and proliferative retinopathy can be found in diabetics who have never received insulin treatment (19), and who are therefore unable to show the presence of humoral insulin antibodies Thus, the presence of cellular autoantibodies must also be assumed here, if pathological changes in the retina and kidneys of diabetics are to be explained on the basis of immunological reactions between insulin and insulin antibodies There are two findings which have kept the discussion open on this topic 1) the demonstration of components in the kidneys, placenta and retina which can bind fluoresceinated insulin (2, 10, 12), and 2) the production of pathological lesions in the kidneys of animals immunized with insulin (38)

Re 1) Demonstration of insulin-binding to pathological lesions in the retina and renal tissue [redacted] almost always done on formalin fixed tissue with fluoresceinated insulin (2, 12) However, the formation of non specific insulin-binding components during the treatment of tissue proteins with formalin and alcohol cannot be excluded (1) Furthermore, during the fluorescence marking of the insulin a change takes place in the insulin molecule, which signifies at any rate a physico-chemical modification, and in many cases also involves an alteration in the immunological characteristics (42) The studies by

Berns et al (2), therefore, at best suggest a binding of insulin to those tissue components in the kidneys which are also outstanding for their PAS-positivity But on the one hand components which bind fluoresceinated insulin do not occur exclusively in diabetic kidneys (22), and on the other hand it is still not proven that these tissue components contain insulin antibodies It has indeed been demonstrated that nodules in nodular glomerulosclerosis (13, 9), thickened basement membranes in the skin (36) and diabetic microaneurisms (13) all contain gamma globulins, but whether insulin is bound to these gamma-globulins in the PAS positive materials, and whether the gamma globulins contain insulin antibodies in diabetics not treated with insulin, is not known It has been shown that in diabetics, those components in the kidneys which bind fluoresceinated insulin far from always bind fluoresceinated anti gamma globulin, which they ought to do provided they were antibodies and thereby immunoglobulins It is more probable, therefore, that insulin is bound to another component in these tissues, possibly a denatured protein In insulin treated diabetics, however, it must be assumed that at the sites where serum gamma globulins can be demonstrated in the tissue, insulin antibodies must also occur, but it is quite uncertain whether nodular glomerulosclerosis *results* from the binding of insulin to sessile antibodies, or from the deposition of circulating insulin insulin antibody complexes Parker et al (42) were unable to demonstrate binding of fluoresceinated insulin to glomeruli of guinea pigs with a high insulin antibody titer This suggests, rather, that the deposition of humoral insulin antibodies in glomeruli of insulin treated diabetics is a sequel of disease in the glomeruli, and not its cause

Re 2) In non diabetic animals injected with crystalline insulin or protamine zinc insulin, it has not proved possible to elicit glomerular changes of a type similar to those seen in diabetic glomerulosclerosis (38, 51) On the other hand, glomerular changes have been described in animals following immunization with insulin emulsified in Freund's adjuvant (38) These changes, however, could also be produced by the injection of Freund's adjuvant alone (38), just as the injection of other substances such as albumin and protamine can give rise to similar changes in the kidneys (17, 30) The changes observed must thus be considered non specific Repeated injections of insulin antibodies into rats resulted in hyperglycaemia and acidosis, but no glomerulosclerosis like lesions (28) There is thus nothing to suggest that the manifestations of long term diabetes in the kidneys and retina are due to insulin–insulin antibody reactions

Other autoimmunological mechanisms

The discussion so far has only been concerned with the possibility that the insulin molecule in diabetics becomes antigenic by a modificaton Other possibilities for the origin of autoimmunity in diabetes mellitus will now be briefly mentioned

It was stated in the introduction that autoimmunological processes can also

arise on the basis of absence of tolerance This requires that the insulin molecule does not circulate through the lymphatic apparatus of the organism until the processes which lead to immunological tolerance have terminated A study of foetal pancreas, however, has shown that beta-cells can be found in only 10-week old foetuses (45), and determination of the insulin in blood specimens from abortions has shown that insulin is present in the blood at a very early stage of foetal life Absence of tolerance is therefore unlikely

An alteration in the function of the immunological apparatus could likewise be considered as causing the autoimmunological processes in diabetes In this connexion, it is of interest that both pernicious anaemia (39) and thyroiditis (21) occur more frequently in a diabetic population than in a normal population, just as in juvenile diabetics in particular, parietal cell antibodies and thyroid antibodies can be found considerably more frequently than in normal subjects (39) It must again be remembered, however, that neither circulating nor cellular insulin antibodies could be demonstrated in diabetics not treated with insulin It is possible that the higher incidence of autoantibodies in diabetics must rather be considered a sequel of the degenerative, vascular changes associated with diabetes, which undoubtedly lead to tissue damage and leakage, and thereby make it possible for certain molecules to come into contact with antibody producing cells, they would otherwise be excluded from In the same way, autoimmunological processes, with renal tissue components as antigen, could be considered to contribute to the pathogenesis of the diabetic nephropathy

For the time being, the concept must be rejected that diabetes mellitus arises, or the complications of long-term diabetes develop, as a result of the formation of autoantibodies against insulin

SUMMARY

The question is examined whether diabetes mellitus can arise, or whether the manifestations of long-standing diabetes can develop, on the basis of auto-immune mechanisms

It is pointed out that no immunological differences have been found between insulin from diabetics and insulin from non-diabetics Nor has it been possible to demonstrate insulin antibodies in diabetics not treated with insulin

However, isoimmunization can occur following subcutaneous injection of insulin in physiological doses without the use of adjuvant It has also been shown that the insulin antibodies formed can react with islet insulin, resulting in histologically demonstrated lesions in the islet tissue It is probable that local tissue reactions and the unphysiologically high insulin concentration at the site of injection are the reason for the injected insulin being regarded as an antigen in these cases

The cause of the lesions described under the designation 'insulitis" in juvenile diabetics is still not elucidated

There is no definite support for the belief that insulin–insulin antibody reactions contribute to the development of the manifestations of long standing diabetes

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THE STATE OF INSULIN IN PLASMA

by

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In recent years, a number of studies have attempted to solve the problems of the biological and physical characteristics of the plasma insulin, and have thrown light on the important question whether insulin exists in more than one form in normal plasma These investigations have been made in part using bioassays, mainly the rat-diaphragm and the rat epididymal fat methods, in part using the immunoassay described by Berson and Yalow or modifications hereof Studies using bioassays have shown the presence in plasma of different forms of insulin like activity (ILA)*), which can be separated in various ways, such as by addition of insulin antibody (anti insulin) to plasma, extraction with acid ethanol, treatment of plasma with cation exchange resin, and electrophoresis However, the methods mentioned have not succeeded in separating different forms of insulin activity which can be determined by the immunoassay of Berson and Yalow It has therefore been maintained that the different forms of ILA found in plasma do not represent different forms of plasma insulin, but other plasma factors with insulin like activity

The present paper attempts to review the studies which have investigated the biological and physical characteristics of the different forms of plasma ILA and of the plasma insulin activity which is measured by the immunoassay

Immunologically active insulin

Insulin is found in plasma in a form in which it can react with insulin-antibody using the technique developed by Berson and Yalow (58) The insulin values found by this and similar methods (28, 31, 43) correspond quite well to the values of "suppressible ILA" (see page 44) found by the rat epididymal fat method (12, 39, 40, 46) Simultaneous determinations of suppressible ILA and plasma insulin measured by the immunoassay have shown identity between the values found with the two methods (53), and it is therefore highly probable that they measure the same form of plasma insulin This form of insulin in plasma has been called "immunologically active insulin' (38)

The physical properties of the immunologically active plasma insulin have

*) ILA the activity which can be measured by a biological method for determining insulin

been elucidated in recent years Electrophoretic studies using starch gel have shown that it moves in the pre albumin zone corresponding to the electrophoretic mobility of insulin labeled with I^{131} (10) Investigations with ultracentrifugation of plasma show that most of the immunologically active insulin is found in the supernatant above the albumin layer, and that insulin I^{131} is distributed in an identical manner (10, 34) Gel filtration studies using Sephadex G 75 seem to indicate that the molecular weight of immunologically active plasma insulin and insulin I^{131} is of the same order of magnitude and lower than the molecular weight of albumin (55)

The investigations cited suggest that the immunologically active plasma insulin probably circulates in plasma in a form in which it is not bound to plasma proteins of high molecular weight, and in which it has the same physical characteristics as pure pancreatic insulin added to plasma

It has constantly been found that the amount of immunologically active insulin in plasma increases after oral glucose administration in normal subjects After intravenous glucose administration the immunologically active plasma insulin increases almost instantaneously 48 , and when the glucose concentration falls the immunologically active plasma insulin decreases, with a half life between 5 and 15 minutes (49, 60) In pancreatectomized dogs, a fall in the immunologically active plasma insulin has been recorded shortly after the operation (22, 50) Studies on the same animal species have shown that the activity of this form of insulin is much higher in the pancreatico-duodenal vein than in the femoral artery, both in the fasting state and after the administration of glucose (40) These results show that the immunologically active insulin is produced in the pancreas, and the evidence suggests that this form of plasma insulin plays a major role in the regulation of the blood glucose level

Non suppressible ILA

Several investigations with the rat epididymal fat method have shown that in normal plasma, only part of the activity measured by this method is inhibited after addition of insulin antibody, which totally suppresses the biological activity of pure pancreatic insulin The plasma ILA which is not inhibited by anti insulin has been called "non suppressible ILA", and the ILA which is inhibited "suppressible ILA"

The physical characteristics of the non-suppressible ILA in plasma have been investigated recently by Froesch and coworkers (Table 1) Electrophoretic studies have shown that the non suppressible ILA is located in alpha2 and beta globulins (13, 24), and gel filtration of plasma using Sephadex indicates that the molecular weight is between 70,000 and 150,000 (13) The group mentioned have succeeded in isolating a substance from plasma which has the same biologic characteristics as non suppressible ILA Their investigations on this "purified non suppressible ILA" have shown that the molecular weight of this substance depends on the pH of the solution in which it is dissolved At acid pH the molecular weight

TABLE 1

Biological and physical characteristics of different forms of ILA

	Biological activity				Inactivation with cystein or gluta thione	Heat inactivation	Extraction with acid ethanol	Anti-insulin inactivation		Electrophoretic mobility	Molecular weight	In experimental diabetes	After intravenous glucose administration
	in vivo		in vitro					Without acid-ethanol extraction	After acid ethanol extraction				
	RD	REF	RD	REF									
ıppres- I A	+ [25]	+ [25]	+ [25]	+ [25]	(+) [13 25]	0 [13]	+ [13 46]	0	0 [33] (+) [46]	beta globulin [illegible] alfa 2 beta globulin [13]	70 000–150 000 [13]	– [46 47] 0 [56]	▮ [39]
n, ex	+ [7]	+ [7]	+ [5 30 51]	+ [30]	(+) [6]		+ [6]	+ [51] (+) [6 30]	(+) [6]	beta gamma globulin [6]	Like albumin [6]		– [5]
ethanol :ts plasma	+ [8]	+ [8]	+ [16 37] (+) [illegible] 0 [8 37]	+ [2 9 33 53]	+ [19]		+		(+) [1 33 50 54] 0 [16 19 34]	Albumin [16 33]	300 000–1 000 000 [16] 7000–14 000 [16 33]	– [20] (–) [40]	+ [20]
of ction			+ [54]	+ [17 31 37]				+ [41 54] (+) [36]		Albumin alfa 1 globulin		– [4]	– [40]
of ction			+ [11 50] ▮ [21]	+ [9 17 21 27 37]				+ [41 54] (+) [36]		beta gamma globulin		0 [4]	0 [21 39]

Abbreviations + positive response (+) incomplete response – decrease ▮ no response

[illegible] 7000, and at alkaline pH 100,000 to 200,000 (24) Froesch and coworkers have suggested that the substance forms polymers at alkaline pH and that the polymers dissociate at acid pH In an attempt to show whether the purified non-suppressible ILA has the same structure as insulin the investigators have treated [illegible] with performic acid, which splits insulin into the A- and B-chain With this method it was not possible to show any A-chain in the purified

non suppressible ILA, but the technique used in these studies was not so sensitive as to exclude the possibility of minute amounts of A chain being present (13)

The biological characteristics of non-suppressible ILA in plasma are not different from those of insulin (Table 1) Both in vitro and in vivo, non suppressible ILA has the same effect on the metabolism of rat diaphragm and rat epididymal fat as insulin (25) Inactivation studies with cysteine and glutathione have shown a partial inhibition of the activity of non suppressible ILA, but treatment with both urea and glutathione inhibits the activity completely Heating to 80° C does not cause a decrease in the activity of non-suppressible ILA (13)

Investigations in normal individuals have shown that non suppressible ILA in plasma is unchanged after intravenous and oral glucose administration (12, 39, 40, 45), and it is so far unknown whether it plays any part in the regulation of the normal blood sugar

Various attempts have been made to elucidate the site where non suppressible ILA is produced in the organism There is evidence suggesting that it is formed in the liver (40, 46) and it has been suggested that non suppressible ILA represents a transformation product of insulin (46) However, liver perfusion studies do not support this theory (35, 52)

In pancreatectomized cats, a slow fall in plasma ILA, probably non suppressible, is recorded after the operation (26), while no decrease has been found in dogs (50) Alloxan treatment of rats induces a fall in non suppressible ILA in plasma (23), and so does infusion of anti insulin (47) Thus, the weight of the evidence supports the theory that intact beta cells are necessary for the production of non suppressible ILA, but it does not prove that non suppressible ILA is a form of insulin

Insulin complex

The terms "insulin complex" and "bound insulin" have been used alternately by Antoniades and his coworkers to describe a substance in plasma with insulin like activity and with the ability to be absorbed on a cation exchange resin (Dowex 50×8) The insulin complex can be eluted from the resin by using an alkaline or acid medium, and if eluted with acid it is inactive on rat diaphragm but not on rat epididymal fat (29) Since the ILA of plasma treated with the cation exchange resin is unchanged after the treatment, it has been concluded that the insulin complex in untreated plasma is without insulin like activity (4) These observations have been confirmed since, in the only publication in which the resin method has been applied to plasma by investigators not taking part in the original studies (51)

In later publications, Antoniades has investigated the properties of the insulin complex (Table 1) In electrophoretic studies it has been found in the beta-gamma globulin area, and after gelfiltration on Sephadex G 100 it appears with the albumin fraction, which proves that the molecular weight is considerably higher than that of the insulin monomer (6)

The biological activity of the insulin complex has been investigated in vivo and in vitro on rat diaphragm and rat epididymal fat, and has been found not to differ from that of insulin (5, 7, 30, 51) The ILA of the insulin complex disappears after treatment with reduced glutathione (6), and addition of anti-insulin results in a partial inhibition of the ILA (6, 30, 51) After acid-ethanol extraction of the insulin complex, the ILA of the extract [redacted] likewise partially suppressible (6) These results justify the conclusion that the insulin complex contains insulin, but not the conclusion that all of its insulin like activity [redacted] caused by insulin

The organ which produces the insulin complex, and the biological role of this substance, is at the present moment unknown Based on very few investigations, Antoniades has suggested that it is produced in the liver (5), but no other studies of this important problem have been published After the intravenous administration of glucose, the same investigator found a sharp decrease in the amount of insulin complex in the serum, and he suggested that a dissociation of insulin from the complex took place (3) No proof of this theory has been presented so far

ILA in acid ethanol extracts of plasma

Several studies have investigated the ILA in plasma treated with acid ethanol, the extraction method which for a long time has been used for the isolation of insulin from pancreas Different modifications of the method have been used, some investigators extracting plasma with hydrochloric acid in ethanol (2, 3, 18), while trichloroacetic acid (TCA) in ethanol has been used in other studies (1, 2, 8, 34)

In the technique used by Davidson and coworkers, the mixture of plasma and acid ethanol is dialyzed against a Gey and Gey buffer immediately after mixing (18) The resulting suspension of denatured protein has a high ILA on mouse diaphragm (18) and rat epididymal fat (44), and ILA is present both in the protein precipitate and in the buffer (18, 44) The ILA [redacted] not suppressed by anti insulin (19) but is destroyed by cysteine treatment (18) The protein suspension does not contain factors which stimulate or inhibit the action of insulin on mouse diaphragm (18) In dogs, Davidson and coworkers found a rapid decline in the ILA of the extracts after pancreatectomy (20) In man, this ILA increases after the administration of glucose (20, 44)

The acid-ethanol extraction method of Scott and Fischer has been used in two studies in pancreatectomized dogs Persistence of plasma ILA which could be extracted with this method was noted in both investigations and both studies showed that a considerable part of the ILA extracted was suppressed by anti-insulin (50, 54)

The technique of using TCA-ethanol for the extraction of plasma was originally used for the isolation of the synalbumin antagonist (57), and several studies have confirmed the presence in the extract of an insulin antagonist active on rat diagragm (1, 2, 32) However, other investigators using the same method did not find antagonistic activ-

ity in the extract, on the contrary they have shown that it has a considerable ILA on rat diaphragm in vitro (14, 33) No satisfactory explanation has so far been presented for these discrepancies ILA has consistently been found in the TCA-ethanol extracts using the rat epididymal fat method (1, 8, 33, 34), and a single investigation has shown that the extract has ILA on both rat diaphragm and rat epididymal fat *in vivo* (8) N-ethylmaleimide, which suppresses the action of insulin on the rat epididymal fat, completely inhibits the ILA of TCA-ethanol extracts (16) Several attempts have been made to suppress the ILA of the extracts with anti-insulin, but the results are confusing Some investigators have shown a partial inhibition of the ILA (1, 33), while no or inconstant suppression has been found in other studies (16, 34)

Kipnis and Stein have drawn attention to the phenomenon that albumin prepared with TCA-ethanol stimulates the action of insulin on rat epididymal fat (34) The nature of this insulin stimulating effect is unknown, but it is of interest that on dilution of the albumin extract, the dose response curve is parallel to that of insulin (16)

Few studies have been published on the physical properties of the ILA in the TCA-ethanol extracts It has been found that the extracts contain immunologically pure albumin, and Sephadex fractionation of the protein indicates a molecular weight over 300,000 (16) After Sephadex fractionation of the extracts, ILA is found both in the fractions containing the high molecular proteins and in the fractions containing substances with a lower molecular weight (16), in contrast to the original observation in which ILA was found only in the latter fractions (33)

The investigations on the ILA of acid ethanol extracts of plasma do not allow any conclusions to be drawn The results are to a large extent contradictory, probably because different modifications of the extraction methods have been used However, it is important to stress that the acid ethanol method of Scott and Fischer extracts a substance from the plasma of pancreatectomized dogs, which biologically and immunologically has the same properties as insulin

ILA in electrophoretically separated plasma protein fractions

Not all investigations of ILA in electrophoretically separated plasma protein fractions have used the same electrophoretic techniques, nor have the methods used for the treatment of the protein fractions after the separation been identical Some investigators have dialyzed the protein fractions against water at room temperature, which increases the ILA of the fractions (36), while other have omitted this step For these reasons, only some of the studies are comparable

Several investigators working with similar electrophoretic techniques have shown that the ILA in serum is distributed with one maximum in the albumin-alpha-1-globulin ("A fraction") and another in the beta gamma globulins ("B-fraction") (17, 21, 37, 56) Although a single observation seems to indicate that the ILA in the B fraction is inactive on rat diaphragm in vitro (21),

most investigations show that the ILA in both the A- and B fraction are active on both rat diaphragm and rat epididymal fat in vitro (9, 11, 17, 27, 37, 56)

Studies using anti insulin have succeeded in showing a suppression of the ILA in both A and B fractions, but while some investigators have found a complete suppression after addition of anti insulin (21, 56) other studies have shown only partial inhibition of the ILA (36)

The biological role of the ILA in the A and B fractions is not known After administration of glucose, no change is noted in the ILA of the B fraction in peripheral venous blood (21, 39) Ten minutes after the intravenous administration of glucose, a fall is seen in the ILA of the A fraction (10), but an increase has been found 30 minutes after oral glucose administration (21) Since the level of the ILA of the A fraction seems to depend on the serum glucose concentration, it is natural to assume that this form of ILA may be of importance in the regulation of the serum glucose Support for this hypothesis may be gained from the observation that a retention of ILA in the A fraction seems to take place in the peripheral tissues (40)

In dogs, the ILA of the A and B fraction has been measured at different sites in the circulation, in an attempt to elucidate where these forms of ILA are produced in the organism (40) This study showed higher ILA of the B fraction in blood from the pancreatico-duodenal vein than in arterial blood The ILA of the A fraction tended to be higher in the pancreatico-duodenal vein than in the artery After pancreatectomy, dogs show a slow fall in the ILA of the A fraction, while the ILA of the B fraction has a tendency to decrease (42) These experiments suggest that the ILA of the A and B fractions is produced in the pancreas

DISCUSSION

The studies which have been cited on immunologically determined insulin and ILA in plasma raise the question whether plasma insulin exists in more than one form The investigations on "suppressible" and "non suppressible" ILA, on "free" and "complex" insulin, and on ILA of "A and B fractions", have separately demonstrated two different forms of ILA in plasma However, both the rat diaphragm method and the rat epididymal fat method which have been used in these studies are non specific, and the demonstration of two forms of ILA in plasma can therefore not be considered a proof that different forms of plasma insulin exist

Using the resin method, Antoniades has isolated the "insulin complex", and several investigators have found that it contains a substance with ILA, which is *suppressed by anti insulin (Table 1)* Since the insulin complex is without any insulin activity measured by the immunoassay (10, 34), the studies show that the insulin complex contains suppressible ILA which is without activity in the immunoassay

Several investigators have found ILA in electrophoretically separated beta-gamma globulin fractions (Table 1)

which have no insulin activity when determined by the immunoassay (10, 59) Since the beta gamma globulins contain ILA which is suppressed by anti insulin, this ILA must be without activity in the immunoassay

It has been found in dogs, that plasma insulin determined by the immunoassay disappears shortly after pancreatectomy (22, 50) However, acid ethanol extracts of plasma from these animals contain ILA which is suppressed by anti insulin (50, 54) Since acid ethanol extraction of plasma causes no increase in the amount of plasma insulin which can be recorded by the immunoassay (28, 34), these investigations show that plasma from pancreatectomized dogs contains suppressible ILA which is without activity in the immunoassay

Thus, three different types of investigations have all led to the conclusion that plasma contains suppressible ILA which is inactive in the immunoassay Besides insulin, no substance is known which has suppressible ILA on the rat epididymal fat pad The studies therefore permit the conclusion that plasma contains a form of insulin which is without activity in the immunoassay, and that at least two different forms of insulin must exist in plasma, one which is recorded by the immunoassay and one which is not These forms of plasma insulin have been designated immunologically active and immunologically inactive plasma insulin (38)

The investigations cited previously raise the question whether the immunologically inactive plasma insulin which is isolated by acid ethanol extraction, resin treatment and electrophoretic separation is an entity, or whether different forms of immunologically inactive insulin exist in plasma Our present knowledge does not permit any definite conclusions to be drawn Although the insulin complex and the ILA of the B fractions seem to have identical biological properties and electrophoretic mobility, their response to intravenously administered glucose is different (Table 1)

Two studies on ILA in electrophoretically separated serum proteins have shown that the albumin alpha 1-globulin fraction, which probably contains the immunologically active plasma insulin, and which has no insulin stimulating effect (41, has an insulin like activity which is much higher than the level of immunologically active plasma insulin (21, 39, 40) These results, and the investigations on the effect of warm dialysis on the level of suppressible ILA in the albumin alpha-1-globulin (36), suggest that in addition to immunologically inactive insulin these protein fractions contain immunologically inactive insulin Thus, the possibility cannot be excluded that two types of immunologically inactive insulin are present in plasma

The physical state of the immunologically inactive plasma insulin has been scantily investigated, but a single observation suggests that it has a molecular weight which is considerably higher than that of the insulin monomer (6) It has therefore been suggested that this form of plasma insulin is in some way bound to a high molecular weight protein If this is the case, it is not surprising that the immunologically inactive plasma insulin does not have any activity in the

immunoassay, and has a different biological half-life from that of the immunologically active insulin (26, 42)

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IODINE-LABELLING IN THE STUDY OF THE BIOCHEMISTRY OF INSULIN

by

K. Brunfeldt

INTRODUCTION

A relationship between diabetes mellitus and the pancreas was first described by the English physician Cawley in 1788 (12) He established that infections of the pancreas that caused atrophy could lead to the development of diabetes

In 1869 the German histologist Langerhans (36) discovered that throughout the external secreting tissue of the pancreas there was scattered a diverging type of tissue This has since been named the islets of Langerhans after the discoverer In man this tissue constitutes approximately 1 per cent of the weight of the pancreas, and the islets are present to the number of 1–2 million

In rodents the pancreas has a diffuse shape and it is therefore possible through a minor incision in the abdominal wall of living mice to exteriorize a lobe of that part of the pancreas that adheres to the spleen This can be performed with preservation of the blood supply On examination under a microscope it can be seen that the islets of Langerhans are richly vascularized, and that the capillaries in the islet tissue have an approximately 50 per cent larger diameter than the capillaries in the surrounding external secreting tissue A plastic injection of the vessels also shows this difference in the diameter of the capillaries (fig 1) (5) It has been known for some time that the islets of Langerhans contain zinc ions, this may be demonstrated by chelate formation with, for example, dithizone (40) The precipitation of zinc dithizonate can be observed in the living specimen after an intravenous injection of a dithizone emulsion (5)

The importance of the islet tissue in the regulation of the glucose concentration in the blood was, as is well known, first demonstrated by Banting and Best in 1922 (2) by the preparation of an active extract of pancreas from dogs where the external secreting tissue was atrophied by ligation of the excretory duct of the pancreas to the duodenum The isolation of the active component, insulin, as crystals was performed by Abel in 1926 (1) The primary structure of insulin (fig 2) was fully elucidated by

*) Given in German as a lecture at the Rheinisch Westfalische Technische Hochschule Aachen West Germany on the 15th of November 1966 by invitation of Deutsches Wollforschungsinstitut

Sanger and collaborators in 1955 (48). The synthesis of insulin was completed in 1963 by Zahn and collaborators (43) and also by Katsoyannis and Dixon and collaborators (33). Fully synthetic crystalline ox insulin was obtained by a Chinese group under the leadership of Wang Yu in 1965 (35).

In the following a synopsis of the biochemistry of insulin is presented in order to indicate the possibilities of the use of iodine labelled insulin (fig. 3).

Biosynthesis

Experiments with minced β cell tissue from Lophius piscatorius are thought to show that the A chain and B chain of insulin are separately synthesized and that the synthesis follows the generally accepted principle for the anabolism of proteins (25). A single chain precursor analogous to, for example, chymotrypsinogen should therefore be out of the question. The combination of the two chains presumably does not require a particularly complicated cellular mechanism, as it is possible *in vitro* to obtain yields of about 50 per cent (30). It is, however, to be regarded as probable that the combination *in vivo* takes place by an enzymatically controlled process. It has been suggested that glutathi



Fig. 1

Plastoid preparation showing the capillary network of an islet of Langerhans and the surrounding external secreting tissue in the mouse (125x).



Fig 2

The primary structure of pig insulin

one-TNP transhydrogenase, which has been demonstrated in the islets of Langerhans, might participate in the process (37)

Storing and secretion

The storing of insulin takes place in the cytoplasm of the ß-cells, possibly in the form of strongly polymerized zinc complexes (41) Apart from the fact that insulin is secreted as a function of the concentration of the blood glucose, the secretory mechanism must be regarded as unknown.

Peripheral processes

Today, dispite a thorough knowledge of the chemistry of insulin, there are still many fundamental questions about the biochemistry of insulin that remain unsolved For example it is not known if insulin be normally transported bound to a blood component Also unknown is its degree of polymerization and thus its real molecular weight in a biological environment (34) It has been demonstrated that insulin can be broken down in the liver by enzymatic reduction of the

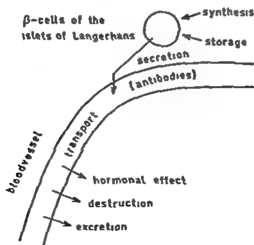


Fig 3

A diagram of the significant biochemical processes of insulin

S S bonds, but whether a special destruction process exists for insulin is not yet known (50). However, the greatest of the unsolved problems lies in the ignorance of the reaction with which insulin carries out its hormonal effect. Many hypotheses have been put forward to explain the mechanism, particularly in connexion with the transport of glucose. But no definite experimental basis is believed at present to give unequivocally a picture of the nature of the action of the mechanism. Similarly, it is not known if insulin carries out its hormonal effect by reacting with one or several receptor systems in the cell.

To a small degree, insulin is excreted in both the normal and the diabetic organism through the kidney (31).

Iodine labelling

In the study of what happens to insulin in the organism, extensive use is made of insulin labelled with the radioactive isotopes ^{125}I and ^{131}I. It should be made clear, however, that a derivative of insulin is thus employed containing a foreign atom. The consequences of this will be illustrated by the examples given in the following.

Even as early as 1936 it had been shown by Harington and Neuberger (23), that it was possible to iodinate insulin with the stable isotope ^{127}I without demonstrable destruction. At the low degree of iodination that is normally used in the labelling of insulin for biochemical investigations the iodine is preferently substituted in the tyrosine residues. In human, pig and ox insulin these are located as A 14, A 19, B 16 and B 26. On the addition of larger amounts of iodine, a substitution also takes place in the histidine residues (45). The histidine residues in the three species mentioned are located as B 5 and B 10. This reaction takes place some 30–100 times slower than the reaction with the tyrosine residues (39). The tyrosine residues can be both mono- and di-substituted in the orthoposition relative to the hydroxyl group (fig. 4). The substitution with the second atom of iodine takes place approximately 30 times slower than the first, calculated from the concentration of the respective phenolate ions (42). Iodination of the phenylalanine residues or formation of thyroxine has not been demonstrated using the mild iodinating methods that are employed in the labelling of insulin.

On the other hand the iodine may be bound to the insulin molecule by non-covalent bonds, which is shown by the following experiment. By iodination of insulin with I_3^- at a given dripping rate and by addition of quantities of iodine corresponding to degrees of iodination of 10, 12, 15 and 20 I/mole, free iodine could only be shown in the reaction mixture with Thio Michler's ketone by the addition of I_3^- corresponding to degrees of iodination above 7 I/mole. After a few minutes' stirring following the completion of the iodination, the reaction for free iodine was on the other hand negative. However, the looser bound iodine was removed by the subsequent gel filtration and drying as the analytical values were 9.2, 9.6, 10.3 and 10.8 I/mole. It is not known how this interaction takes place, but bonding by inclusion seems to be a theoretical possibility.

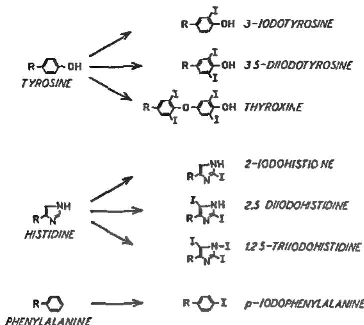


Fig 4

Substitution possibilities for iodine into various groups of the insulin molecule

Generally, the iodination is performed at an alkaline pH, in that the reaction with the tyrosine residues is an electrophilic substitution certainly involving a quinod intermediate The I^- that is released by the reaction is separated from the protein together with the other low molecular components of the reaction-mixture by gel filtration on Sephadex G 25 In gel filtration the insulin may be completely dezinked (7) The buffers used for the elution in the gel filtration of micro-quantities must contain 1 per cent albumin in order to avoid adsorption onto glass and column material The presence of the albumin also reduces the radiation – induced damage of the insulin By monitoring the radioactivity in the effluent from the column a simple method is obtained for investigating if the iodination has taken place stoichiometrically correct and if any artefacts have occurred This ought always to be carried out in connexion with iodination on a microscale with carrier free isotope solutions This is because the production of these is still not under complete control, which could discernibly influence the iodination

The first radioactive iodine isotope that became generally accessible was ^{131}I This isotope radiates a powerful γ-component of 364 Kev (82 per cent) and a β^- with an energy of 610 Kev (87 per cent) whereby ^{131}I is transformed to $^{131}_{54}Xe$ Because of this high energy the isotope is easy to trace, this is important if scanning, for example, is to be carried out after a paper electrophoretic fractionation Half life is, however, only eight days and, therefore, the labelling process must be repeated often

It should also be remembered that "carrier free" preparations of this isotope also contain ${}^{127}I$ and ${}^{129}I$ The relative abundancy of the three isotopes [redacted] approximately 20, 20 and 60

Nowadays, ${}^{125}_{53}I$ is generally employed in labelling this is [redacted] pure γ emitter with an energy of 35 Kev (100 per cent) and a half life of 60 days Degradation takes place by electron capture to ${}^{125}_{52}Te$ over the metastable ${}^{125m}_{52}Te$ (X ray 27 Kev) The measurement of these relatively weak energies will, with the use of a scintillation system with a wellcounter, cause but little difficulty

As a rule the isotope [redacted] supplied as I^- in a weak alkaline solution and various methods have been used for the oxidation to I As a high specific activity is normally required the aim is that as large [redacted] part of the isotope as possible reacts with the protein Oxidation to iodine can take place by the addition of the isotope to an I_3 solution This method is, however, not particularly suitable as it is essential to have an excess of I^- to ensure that I is held in solution With a ratio of I_2/I of 1 4 only 1/6 of the radioactivity will be incorporated in the protein Iodination with an I_3 solution is mild and if a low specific activity [redacted] required this [redacted] the method that [redacted] applicable In another method that was earlier much used I^- is oxidized with IO_3^- and the released iodine extracted with CCl_4 or $CHCl_3$ that is slowly added to an aqueous solution of insulin whilst stirring vigorously By this method it is possible for theoretically half of the added radioactivity to be used in the labelling of the protein In iodination with small quantities of iodine the yield is, however, reduced considerably (6) In addition, the strong emulsion formation together with the high volatility of the iodine and the non polar solvent make this method less suitable

Where high specific radioactivity is required the method most used today is that of Hunter and Greenwood (1962) (26) Oxidation of I^- takes place with an excess of chloramine-T, so that a large part of the I^- that is released during the substitution process [redacted] reoxidized, and so up to 90 per cent of the radioactivity [redacted] incorporated in the insulin molecule









$$[(CH_3-C_6H_4-SO_2NCl)^- + H_2O \rightleftharpoons CH_3-C_6H_4-SO_2NH_2 + ClO^-,$$

$$ClO^- + 2I^- + 2H^+ \rightarrow Cl^- + H_2O + I_2]$$

The iodination [redacted] carried out in the ampoule in which the radioactive isotope is delivered in a volume of 10–20 µl The iodination normally takes place on 10 µg insulin dissolved in 50 µl The reaction time after the addition of chloramine-T is 1 minute The oxidation is halted by sodium disulphite ($Na_2S_2O_5$), whereby any possible excess of I_2 is converted to I^- The use of an oxidation agent involves the possibility of destruction, but carried out correctly this method gives excellent results for the iodination of insulin This is possibly because the SH group [redacted] not found in this protein In order to avoid the use of an oxidation agent electrolytically liberated iodine has been employed (47)

Location of iodine in the insulin molecule

Fraenkel Conrat and Fraenkel Conrat (1950) (18) have shown that the method of iodination is of importance for the distribution of the iodine in the insulin molecule This was demonstrated by iodinating to nearly the same degree using two different procedures A difference in the amount of monoiodo- and diiodo-tyrosine residues appeared as well as a difference in the biological activity

A Iodination with an excess of I_3^- and halting the reaction with disulphite
B Iodination by slow addition of I_3^-

	TYR	MIT	DIT	% Biological activity
A	44	23	33	27
B	50	32	18	67

de Zoeten (1959) (54) has shown that the iodine is not equally located in the four tyrosine residues After sulphite cleavage the A- and B-chains were separated by paper electrophoresis The A-chain was cleaved by chymotrypsin between tyr14 and glu NH$_2$15, and the B-chain by trypsin between arg22 and gly23 By these means the four tyrosine residues A14, A19, B16 and B26 were located each in its own chain fragment. By paper electrophoresis the chain fragments were then separated, and the iodine content determined by measurement of the radioactivity from ^{131}I With the help of this tecnique it could be shown that by iodination with I_3 at pH 9 2 to 0 2–3 I/mole the iodine was incorporated to a higher degree in the A-chain than in the B-chain (55) By complete hydrolysis with pancreatin and chymotrypsin it was shown that the content of diiodo-tyrosine also was considerably greater in the A-chain than in the B-chain Of the individual tyrosine residues B16 was the least reactive (56) These studies were developed by others investigating the distribution by iodination at other pH values By iodination to 0 7 I/mole at pH 1–5 only about 10 per cent of the iodine was incorporated in the B-chain, whilst at pH 9–11 15–45 per cent in the B-chain was shown B26 in particular showed a large increase in reactivity while B16 was still of low reactivity even at pH 11 (14) It was also shown that iodination in 8 M urea reduced the difference in reactivity of the four tyrosine residues An equality of reactivity was, however, first achieved after the splitting of the S-S bonds by performic acid This indicates that a certain folded structure exists even in 8 M urea (15)

After iodination of 10 µg insulin at pH 8 6 by the method of Hunter and Greenwood, it could be shown that a larger part of the iodine, about 25 per cent, is incorporated in the B-chain by iodination to about 0 7 I/mole than by iodination with I_3^- (27) This indicates that this method is perhaps less mild than the previously mentioned where iodine is slowly added or liberated

Iodination under special conditions with ICl has been performed at pH 1 where insulin has the tendency to form fibrils Here, 6 I/mole at the most could be taken up, even on the addition of a quantity of iodine equivalent to 20 I/mole Moreover, on iodination with up to 1

l/mole almost all of the iodine was incorporated in the A chain It is presumed that fibrillation is involved and protects one of the B-chain's tyrosine residues under these conditions of iodination (49)

Influence of iodination on the chemical properties of insulin

Formerly one was not aware of the radical alterations which iodination could bring about in the chemical properties of insulin The electronegative properties of iodine and the size of the electron shell (53 electrons) should be kept in mind as well as the size of the iodine atom, which is nearly equal in size to the benzene ring (fig 5)

The electronegative properties of iodine can easily be shown by the decrease in the pK value of the phenolic OH-group that is brought about by substitution in tyrosine In free tyrosine pK is 10 1, by mono-substitution it is reduced to 8 2 and by di substitution to 6 5 (24), (20) The pK value of the phenolic OH groups in the tyrosine residues of the insulin molecule is somewhat increased because of the proximity of other negative charges So three of the tyrosine residues have a pK value of 10 4, whilst the fourth, which is probably hydrogen bonded, has a pK value of 11 4 (28) In accordance with these increased values in relation to free tyrosine, a mean pK value for the di substituted tyrosine residues of 7 9 has been determined from measurements of insulin with a degree of iodination of 8 I/mole (21) As all tyrosine residues are not di substituted at this degree of iodination, this determination of pK value is probably too high

The effect of the substitution by iodine is not, however, restricted to the phenolic OH group, in that it is also possible to show an electronegative effect on the protons in orthoposition to the iodine By proton magnetic resonance (PMR) measurements, a downfield displacement can be shown of similar size for the absorption frequencies for the 2 protons in monoiodo-tyrosine as well as the 2- and

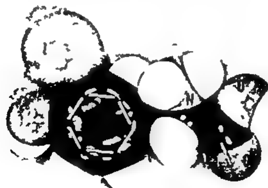


Fig 5

Stuart Briegleb model of 3 monoiodo L-tyrosine

E protons in diiodo-tyrosine The measurements were carried out on the amino acids dissolved in trifluoroacetic acid (fig 6) (7)

The UV absorption of tyrosine alters greatly with iodination, in that the absorption is shifted towards the longer wave lengths and is increased For pH values where the dissociation of the phenolic OH group begins to take place, the absorption will shift towards the longer wawe lengths aswell for nonsubstituted as for substituted tyrosine The alteration in the absorption can be used as an estimation of the degree of iodination (7) This is shown by measurements of the absorption spectra of iodinated insulin preparations (fig 7)

Iodine substitution possibly exerts an influence on the conformation of the insulin molecule as a result of the size of the iodine atom and/or by the reduction of the pK value of the phenolic OH-groups The increased dissociation capacity especially by di substitution would thus mean that the hydrogen bonds which the tyrosine residues are involved in, if at all will break at a physiological pH Measurement of circular dishroism seems to show that the iodination exerts an influence on the conformation of the insulin molecule In non iodinated pig insulin a degree of helicity of 26 per cent was found, while in dezinked insulin 19 per cent and in insulin iodinated to 4 and 8 I/mole 12 per cent was demonstrated at pH 9 (10)

A relationship between zinc and insulin has long been acknowledged because of the ease with which insulin crystallizes with Zn^{++} as rhombohedral crystals The bonds between Zn^{++} and insulin are, however, relatively weak Thus the insulin and Zn^{++} are separated completely or partly by electrophoresis in certain commonly used buffer solutions (7) (fig 8)

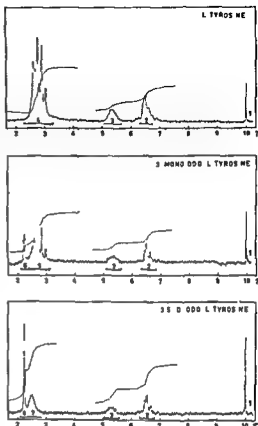
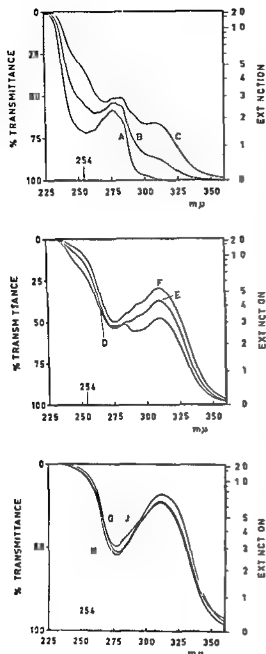


Fig 6

PMR spectra of A) L tyrosine 95 mg in 500 µl trifluoroacetic acid (TFA) B) 3 monoiodo-L-tyrosine 95 mg in 700 µl TFA B) 3 5 diiodo-L-tyrosine 142 mg in 700 µl TFA Reference [illegible] µl tetramethylsilane Signals 1—Reference 2—CH_2 3—CH 4 and 5—Aromatic protons and NH_3^+ 6—Protons in orthoposition relative to I (2 protons) 7—NH_3^+ 8—Protons in orthoposition relative to I (2 and 6 protons)

Iodination, particularly at the higher degrees of iodination, clearly diminishes the capacity of insulin to bind Zn^{++} In fig 9 this [illegible] shown by paper electropho-



F g 7

UV al n ectra of amorphous insul n A an xi a d nsul ns B–J) n TRIS—bor c a l u f pH l Iod ne content (gram atoms pe n ul n B 1 C 2 D 3 F 4 F 5 (H 7 J 8 Concentrat on 0 04 μM ml

resis in a barbiturate buffer at pH 9 of iodinated insulin with addition of ^{65}Zn containing ZnCl The added quantity of Zn was equivalent to the normal content in crystalline insulin that is 0 5 per cent The effect begins to make it self felt at 3–4 I/mole, then eventually at a degree of iodination of 9 6–10 3 I/mole the capacity to bind Zn is completely lost If the imidiazole groups of the histidine residues are destroyed by photo-oxidation at 8° C with methylene blue as photosensitizer the insulin completely loses the capacity to bind Zn + by electrophoresis under the above mentioned conditions On the other hand a carbamylation of the N terminals seem to be of little or no importance This shows that especially the imidazole groups of the histidine residues are of importance for the binding of Zn+ Iodination of the histidine residues is therefore probably the reason for the reduced capacity of the iodinated preparations to bind Zn (10)

Effect of iodination on biological activity

A decisive complication for the use of iodinated insulin in biological experiments is the reduction of the hormonal effect even at low degrees of iodination By intravenous injection in rabbits it was demonstrated that 2–3 per cent of the original effect remained by iodination to 10 3 I/mole but no effect at 10 8 I/mole By measurements of the glucose utiliza tion on isolated fat cells of rats it was demonstrated that a reduction to 1 per cent of the original activity took place at 4 I/mole and only a trace of the activity at 5 I/mole (9) The isolated fat cells were produced from

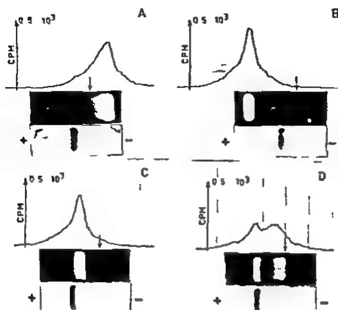


Fig 8

Agar electrophoresis of ^{65}Zn labelled pig insulin in different buffers A – TRIS boric acid pH 9 1 B – TRIS boric acid EDTA pH 8 9 C – Glycine NaOH pH 10 0 D – Barbiturate pH 8 6 The agar gel was prepared by mixing equal parts of a 2 % agar solution and the respective buffer solution Sample 0 125 mg insulin in 5 μl volume Voltage 120 volts Duration 75 min Temperature 22 C Fixation by drying Stain Amido Black – From top to bottom Record of radioactivity (Geiger tube) Autoradiograph (Codirex X ray plate exposure 7 days) Agar electrophorogram Application point is marked by an arrow

adipose tissue of rats by treatment with collagenase (46), (19) In principle the method is the same as that in the fat pad method, in that the quantity of ^{14}CO from ^{14}C-glucose is taken to be an indication of the hormonal activity The fat cell method is, in comparison with other biological methods, very precise and allows a large number of measurements per working dag

The difference between the results of the two methods might mean that in the intact organism some de iodination took place However, activity has been demonstrated (29) at degrees of iodination higher than 4 I/mole with the rat fat-pad and rat diaphragm methods An unspecificed adsorption onto the surface of the cells could not be the reason for the low activity shown by the fat cell method as the addition of a considerable quantity did not produce a large increase of glucose utilization The reason seems to be, therefore, that the surfaces of the fat cells are altered by the collagenase treatment so that they react different-

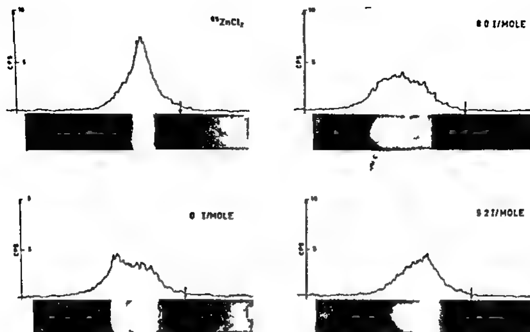


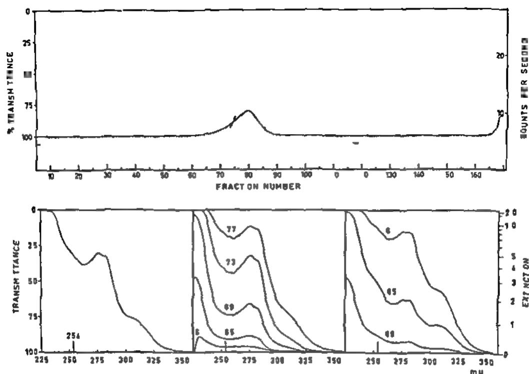
Fig 9

The effect of iodination on the Zn++ binding capacity of insulin estimated by paper electrophoresis with addition of ZnCl From top to bottom Record of radioactivity Autoradiograph (Gedurex X ray plate exposure 14 days) Paper electrophorogram Application point is marked by an arrow

ly to a derivatization of insulin from the way in which they react in the isolated tissue or intact organism

The reduction of the biological effect following iodination is in itself no proof that the tyrosine residues are the active centres in the insulin molecule It is thus possible that the iodination of these involves alteration of the conformation—perhaps due to a breakdown of external hydrogen bonds— and that this alteration of conformation restricts the insulin from reaching the cellular receptor or from reacting with it The altered charge values due to iodination might also be a cause The high iodinated preparations do not seem to react at all with the cellular receptor, for with the above mentioned intravenous injection in rabbits no hyperglycemia with quantities equimolar to 40 µg (1 unit) crystalline insulin per kg has been traced

Even at low degrees of iodination the reduction of the biological activity complicates the interpretation of the experiments aimed at illuminating the distribution of insulin in the organism This is partly because the distribution of iodine between the individual molecules is not uniform and partly because the ease of tracing the molecules is proportional to the number of incorporated iodine



F g 10

Dens y grad ent electropho es s of 29 mg 127 131 I nsul n 1 I/mole TRIS bor c ac d buffer w h sucrose as grad ent mater al pH 9 0 250 volts 8 mamp n 65 hours at 25 C
D ect on of m grat on left to a r ght e upwards in the column
A) By emp ying the column the effluent was mon tored for UV absorpt on at 254 m us ng an LKB 4700 UV cord and for rad oact v ty us ng a Ge ger tube —— UV absorpt on rad o- ac ty
B) UV absorpt on of the or ginal ample 520 μg/ml at pH 9 0 (left) and pa of adjacent 2 ml fractions of effluent (m ddle and r gl t) The number on tl e curve s the lower frac on number of the pa r of f act ons pooled Buffer w thout sucro e and fract ons nos 99 100 were u ed re spect ely as references for tl e or g nal sample and the fract ons

atoms i the molecules with the most altered biological properties The heterogeneous distribution of iodine must be expected because of the many substitut ion possibilities 80 (i e 3^4–1) based on the four tyrosine residues It can be shown that there really heterogeneity by density gradient electrophoretic frac tionation at pH 9 of a preparation conta ning 1 I/mole (fig 10) The iodi nation was undertaken by slowly drip ping in an I_3 solution at 0° C and with vigorous stirring so that distribution as uniform as poss ble was achieved The *relatively high UV absorption at 311 mμ* of the most rapidly migrating fractions as against the less rapid shows that the iodine content larger in the more rapid fractions The larger iodine content of these fractions is also indicated by the higher specific radioactivity of these (7

In vivo *experiments*

[1] I labelled insulin of low degrees of

iodination has been used for tracing the rate at which insulin disappears from the site of injection (4).

Iodinated insulin has also been used to investigate the distribution of insulin in different organs. Experiments with iodinated ribonuclease seem, however, to be distributed in a similar manner to insulin (17).

By perfusion experiments it was shown that insulin of low degrees of iodination was not demonstrably removed in its passage through the liver, whilst this was markedly the case with insulin iodinated to 8 I/mole (38). The distribution of ^{131}I-labelled insulin in striated rat musculature has recently been investigated. Rat femoral musculature was incubated in a Krebs-Ringer bicarbonate solution containing ^{131}I labelled insulin. An association to sarcolemma was shown, however there is no certainty that an iodinated insulin preparation that was homogenous and that had full biological activity was used, nor can artefacts deriving from unspecified binding be excluded (16).

Immunological properties

Berson et al. (1956) (3) and Welsh et al. (1956) (51) showed that ^{131}I labelled insulin was eliminated more slowly from the circulation of diabetic patients who had been treated with insulin than from nontreated subjects. The reason proved to be the presence in the blood stream of antibodies directed against insulin.

Formation of antibodies after treatment with insulin preparations had already been shown, but it was believed that the antibodies were produced by the more highly molecular proteins that were to be found as contamination in insulin preparations. However, it is now known with certainty that even the insulin molecule can have antigenic properties when the difference in the amino-acid composition between the homologous and the heterologous insulin is of the same order as between ox- and pig insulin. On the other hand, it must still be regarded as uncertain whether a difference in a single amino acid or immunization with insulin from the same species can occur. A weak admixture of species foreign insulin or an alteration in some of the insulin molecules during the preparative process is possibly the cause of the positive results that have been achieved. Whether auto-immunization is a possible cause of diabetes mellitus must be regarded as a completely unanswered question.

The detection of antibodies takes place by the addition of ^{125}I- or ^{131}I-labelled insulin to serum and by a separation of the free from the antibody bound insulin after it has been allowed to stand. This separation was originally undertaken by paper electrophoresis alone or combined with chromatography. The distribution of the labelled insulin was determined by scanning of the radioactivity. Berson and collaborators showed in their work that iodine labelled insulin and unlabelled insulin compete for the binding to the insulin antibodies. This observation formed the foundation for the quantitative method, which was developed for the determination of the physiologically occuring quantities of insulin in plasma, Yalow and Berson

(1959) (52), Yalow and Berson (1960) (53) By addition of increasing quantities of non labelled insulin it was demonstrated that decreasing quantities of labelled insulin were bound to the antibodies By comparing the values thus found with the competitive effect of an unknown sample, the concentration of the insulin could be determined

The electrophoretic separation procedure are, however, elaborate and the measurements of the radioactivity that must be made by integration of the scanning curve is time-consuming and not particularly exact It is, however, possible to make a separation of free insulin and antibody bound insulin by adding a precipitating antiserum reacting with the insulin antibodies and perform the separation by centrifugation or filtration through microporous filters (fig 11), Morgan and Lazarow (1962) (44), and Hales and Randle (1963) (22) It is easy to measure the radioactivity retained on the filters by placing them in test tubes and using a scintillation well counter system By adding increasing amounts of the non labelled insulin, thus diminishing the antibody-bound radioactivity, a standard curve of the shape shown in fig 12 is obtained By taking the maximum reactive part of ^{125}I- or ^{131}I insulin as 100 per cent, the accuracy expressed as SEM of duplicate determinations is on an average found to be ± 0.6 per cent precipitated radioactivity, by count
m normally produced by immunizing
ing 10^4 pulses The insulin antiserum is normally produced by immuniting guineapigs, and the precipitating antiserum by immunizing rabbits with a γ globulin preparations of guinea pig serum

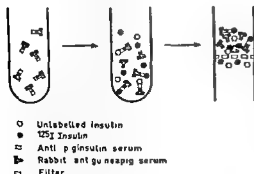


Fig 11

The principle of the double antibody immunoassay using preprecipitating technique

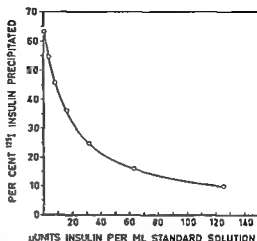


Fig 12

Standard curve for immunoassay of insulin obtained by the addition of known amounts of unlabelled pig insulin Pig insulin antiserum was used for insulin binding

The use of yet another immunological reaction increases the possibility of error, This can, however, be avoided by simple precautions (8) One source of error in the determination of insulin in plasma is that a cross-reaction may take place between the precipitating antibodies and the γ globulins in plasma or serum whose insulin concentration is to

be determined. If such a cross-reaction occurs, some of the precipitating antibodies are taken up. At lower concentrations of the precipitating antiserum this is alleged to cause a low yield of the insulin – insulin antibody complex, resulting in insulin values that are too high (22). In our laboratory we have not been able to confirm this observation, but found that the cross-reaction set in at high concentrations of the precipitating antibody. This results in an increased precipitation due to a decrease in the excess of the precipitating antibody. The reason for this result is that the formation of the soluble complexes between the insulin antibodies and the precipitating antibodies, because of the excess of the latter, is reduced. Disagreement between the results of the two investigations may be due to individual differences between the precipitating antisera employed. The influence of the cross-reaction on the results of the precipitation can, however, be avoided by allowing the insulin antibodies to react with the precipitating antibodies before reacting with the insulin (22). The reaction between the insulin antibodies and the precipitating antibodies has no effect on the reaction of the insulin antibodies with insulin. The distance between the bonds to the insulin and the precipitating antibodies thus seems to be large.

A second source of error in the double antibody method is the influence of complement on the immunological reactions. By pre-precipitation the complement can restrict the reaction between the precipitated insulin antibodies and insulin, resulting in artificially increased values of the insulin concentration. If pre precipitation is not used, the complement will directly influence the precipitation reaction. The influence of the complement is completely negated by the addition of a heparin preparation with anti complement effect, by lengthy storage at $-20°$ C or by dilution (8). Addition of EDTA will also suspend the effect (44).

Because of the extreme exactitude of the double-antibody method it is possible to examine the influence of derivatization on the reaction between insulin and insulin antibodies even when the alterations in the insulin molecule are small. It has thus been possible to examine the influence of iodination on the reaction between insulin and insulin antibodies at degrees of iodination from 0–10.8 I/mole. At a degree of iodination of 2 I/mole a reduced reactivity with the insulin antibodies could be traced from the extent to which ^{127}I substituted insulin was able to compete with ^{125}I-insulin with a degree of iodination of about 0.7 I/mole. The effect of the increasing degree of iodination was progressive, but even at a degree of iodination at which the histidine residues were iodinated to a considerable extent, it could be shown that insulin was reacting, though only weakly, with insulin antibodies (9).

Determination of insulin in plasma and urine

In the following, some examples will be given of the use of the double-antibody method for the determination of insulin in plasma and urine. The examples given are from investigations carried out in our laboratory (32) and (31). The

variation in the concentration of plasma insulin in six normal persons to whom 1 g of glucose per kg of body weight had been administered perorally can be seen in fig 13 It will be observed that a parallel can be traced between the con centration of the insulin and the con centration of the glucose Another ex ample, fig 14, shows the concentration of plasma insulin in a non diabetic obese patient to whom 80 g of glucose had been administered perorally It will be observed that an even steeper rise—to 13–1400 µunits/ml—takes place When the diet was reduced to 1000 kilo-cal *per diem* a clear reduction of insulin se- cretion could be detected

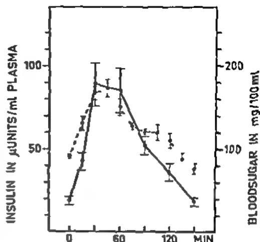


Fig 13

Blood glucose - - - - and plasma insulin —— concentration in 6 normal persons during oral glucose tolerance test 1 g d glucose was ad ministered per kg body weight

In a determination of the concentra tion of plasma insulin during a glucose-tolerance test on a newly diagnosed juve- nile diabetic, a certain insulin concentra tion was shown, but here the administra- tion of glucose only caused a very slight increase in the insulin concentration, fig 15 In this patient the insulin concentra tion was sufficient for the disease to be treated for a while by diet Later, how- ever, the insulin secretion was reduced further and insulin treatment became necessary

That diabetes embraces at least two

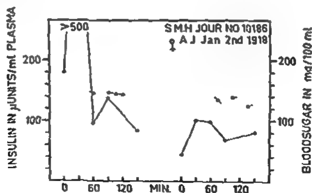


Fig 14

Blood glucose - - and plasma insulin ——concentrations in an obese non diabetic patient during oral glucose tolerance test before and after restriction of diet to 1000 kilo-cal per day 80 g of d glucose was administered Weight before food restriction January 13th 1964 133 8 kg and after January 27th 1964 129 6 kg

different forms of disease may be seen from comparison of fig 15 and fig 16 In the latter the concentration of plasma insulin is plotted for an elderly, somewhat overweight diabetic patient receiving glucose In this case a somewhat increased fasting level value and a continuing rise of both the concentration of insulin and the concentration of blood glucose can be shown.

It is interesting to note the difference between intravenous and peroral administration of glucose in their effect on insulin secretion, fig 17 In the case of intravenous injection only a slight and brief increase was observed, whereas the peroral administration brings about a considerable increase in secretion This seems to indicate that when glucose is assimilated from the intestines a factor is liberated which increases the secretion of insulin, and this may possibly be of a hormonal character

The double-antibody method may also be employed for the determination of the insulin concentration in urine With eleven normal persons it could be shown that when they were on a non-standardized diet they had an average secretion of 14×10^3 μunits every 24 hours In normal subjects a stimulation of the endogenous insulin production could also be detected under glucose administration For diabetics a noticeable increase could be observed after treatment with insulin (31)

The great sensitivity with which it is possible to determine alterations in the reaction with insulin antibodies as a result of derivatization may also be exploited in investigations as to whether there is a difference between the extracted and the circulating insulin If such a difference exists, the standard curves for the two insulins will not be identical, since the reaction with homologous antiserum will be stronger Whereas the results plotted for the dilution series of the insulin that is used for immunization as a function of the expected concentration will give a rectilinear function, a divergent structure of the

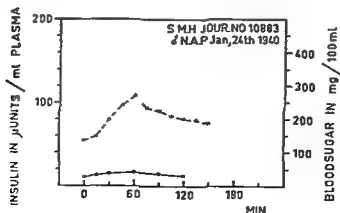


Fig 15

Blood glucose -- ar d plasma insulin —— concentrations in a juvenile diabetic patient during oral gluc t lerance test. 1 g of d glucose was administered per kg body weight

antigen will result in an upward convex curve. By employing this method it was shown that at least the bulk of the circulating and immunologically demonstrable insulin in normal obese and diabetics subjects is identical with extracted

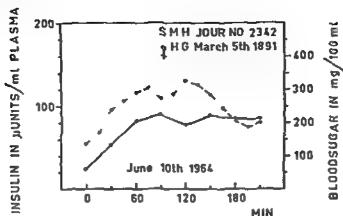


Fig 16

Blood glucose and plasma insulin —— concentrations in a maturity onset stable diabetic patient during oral glucose tolerance test 1 g of d glucose was administered per kg body weight

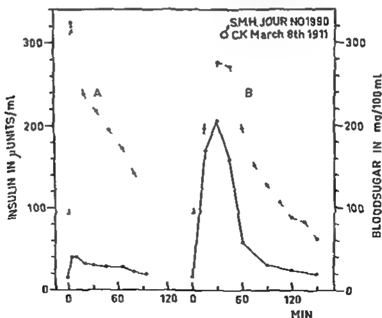


Fig 17

Blood glucose and plasma insulin —— concentrations following A) intravenous injection of 100 ml 25% d glucose and B) peroral administration of 1 g d glucose per kg body weight in a patient on whom has been performed vagotomia et operatio plastica pylori

human insulin (13). It could also be shown that crystallized insulin extracted from the pancreas of stable onset diabetic patients and non-diabetic patients showed immunological identity (11).

CONCLUSION

Even though labelling of insulin with radioactive iodine isotopes has increased our knowledge of what happens to insulin in the organism, there still remains a great unanswered question concerning the working mechanism of insulin. While little decisive progress has been made in this area with the employment of iodine-labelled insulin, it is possible that this is due to the alteration which derivatization brings about, and the heterogeneity with which iodination takes place. The use of an insulin incorporating ^{3}H bound to carbon, or ^{14}C or ^{35}S in predetermined positions in the insulin molecule is naturally a considerably more attractive proposition for a biochemist than the use of an insulin derivative in which labelling is carried out by means of an atom that is not originally found in the insulin molecule.

The possibility of producing well-defined derivatives also affords exciting prospects for further research into the working mechanism of insulin. It is therefore not surprising that the attention of the scientific world is directed towards those centres in which research on the organic-chemical synthesis of insulin is being carried out. *Professor Helmut Zahn* and his collaborators occupy a prominent position in this field, not simply because of the scientific results achieved but also because of the freedom with which these results are made available for further advances.

Let us finally return to the point of departure of this lecture: the disease diabetes mellitus. Of the 800 million people who live in Europe and the USA, an estimated 1.3 million suffer from this disease to such a degree that they must be treated with insulin. The serious complications that so often, unfortunately, accompany this diseace, such as retinopathy–which may lead to blindness–, and nephropathy–which can destroy completely the function of the kidney–, urge us on to make greater efforts to solve the two fundamental problems: the cause of the disease, and the mechanism of the hormonal effect of insulin.

Our enthusiasm at our knowledge of the insulin molecule may however have seduced us into concentrating upon this single molecule. In our present state of knowledge there seems to be no doubt that hereditary predispositions lie behind the occurrence of the disease, and we should, therefore, perhaps turn our attention away from the insulin molecule and the reactions directly linked with it to the more central processes in the function of the ß-cell of the islets of Langerhans.

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INSULIN ACTION ON THE CENTRAL NERVOUS SYSTEM

by

Ole J Rafaelsen

INTRODUCTION

This paper is concerned with the evidence of the *direct* action of insulin on the central nervous system It is *not* concerned with the *indirect* action of insulin on brain *secondary* to insulin action elsewhere leading to changed composition of metabolite content in the arterial blood sent to the brain, the most obvious change being a reduction in the arterial glucose offered to the central nervous system Furthermore, this paper is not concerned with the evidence for a diabetic encephalopathy or with the consequence of prolonged and/or repeated hypoglycemia as demonstrated by neuropathological examination Insulin action on the peripheral nervous system and peripheral diabetic neuropathy will only be touched on for comparison

The topic has been reviewed elsewhere, including the problem of a direct or an indirect action of a hormone on a target tissue (32, 34) I have chosen here to tabulate some of the pertinent findings, and I will focus the discussion on some recent findings which have added much to an understanding of this problem

In 1961 Chowers, Lavy and Halpern (5) from Israel reported that insulin administered intracisternally in dogs caused a fall in glucose content both of the cerebrospinal fluid and of the blood The fall was noted during the first 30 minutes after the insulin injection and was a maximum 90 to 120 minutes after administration of the hormone The reduction was both absolutely and relatively more pronounced in cerebrospinal fluid (50 mg per cent) than in blood (36 mg per cent), and it is also lasted longer before preinjection values were regained in cerebrospinal fluid glucose content than in blood glucose This finding indicated to the authors a direct effect of insulin on brain structure In the same study they injected radioactive insulin labeled with ^{131}I intracisternally, and the investigators were unable to detect radioactivity in peripheral blood during the first 6 hours after introduction of labeled insulin intracisternally It was concluded that insulin was unable to pass the barrier between cerebrospinal fluid and blood, and that the fall in systemic blood glucose values was mediated via insulin action on some brain structure It was suggested that insulin in these experiments stimulated para-

SOME STUDIES OF INSULIN ACTION ON THE CNS

A Animal tissues in vitro

Preparation	Parameter studied	Investigators	Comments
Brain slice (rat)	Glucose uptake 10 % increase	Rafaelsen (1961 b)	Only in 'first' brain slice and with high insulin conc
do	*Glucose conversion to carbon dioxide no effect*	*Beloff Chain et al (1956)*	
do	Glycogen formation no effect	McIlwain & Tresize (1956)	
do	Glucose incorporation into lipid no effect Acetate incorporation into lipid 100 % increase	Grossi et al (1962)	
Anterior pituitary (calf & rat)	Glucose uptake 25 % increase Alloxan diabetes reduced glucose uptake normalized by insulin *in vivo*	Goodner & Freinkel (1961)	
Spinal cord (rat)	Glucose uptake 25 to 100 % increase Alloxan diabetes reduced glucose uptake normalized by insulin *in vitro*	Rafaelsen (1958 1961 a)	Insulin effective in conc down to 100 µU/ml
do	Glucose conversion to carbon dioxide and glycogen formation no effect	Rafaelsen & Clausen (1961)	
do	Glucose incorporation into lipid no effect Acetate incorporation into lipid 150 % increase	Rafaelsen Adams & Field (unpublished)	

sympathetic centers in the brain stem, leading to vagal stimulation of the pancreas subsequent insulin release followed by increased glucose uptake in the periphery and thereby fall of blood glucose values The authors thus assumed that the effect of insulin after intracisternal application was a *direct* effect on glucose uptake in the central nervous system and *n indirect* effect via vagal stimulation on the rest of the organism The same authors have in 1966 (6) added supporting evidence to this hypothesis by performing the same type of experiment on vagotomized dogs, where again a significant fall in glucose values in cerebrospinal fluid was obtained, but now only a slight, insignificant fall in blood glucose values

The importance of these experiments in a broader view is closely connected with the question whether insulin does

B Infusion and perfusion studies

Preparation	Parameter studied	Investigators	Comments
Infusion of hexoses and pentoses (rat)	Distribution volume in brain no effect	Park et al (1955 1957)	
Infusion of hexoses and pentoses (cat)	Distribution volume in brain increased	Sacks & Bakshy (1957)	
Perfusion of isolated brain (cat)	Glucose uptake no or slight increase	Geiger et al (1954 1956) Allweiss & Magnes (1958)	
Cerebral arterio venous difference (homo)	Glucose disappearance no increase	Himwich et al (1941)	Interpretation difficult as cerebral blood flow was not determined
	Glucose disappearance up to 50 % increase	Genes (1961)	
Cerebral blood flow determination (Kety Schmidt or modifications) (homo)	After insulin alone decrease of cerebral glucose uptake	Kety et al (1948 a & b) Gottstein et al (1965)	
do	Demonstration of glucose threshold in brain which is insulin sensitive	Butterfield et al (1966)	
do	After glucose infusion Slight increase of cerebral glucose uptake After glucose insulin infusion 50 % increase of cerebral glucose uptake	Gottstein et al (1965 1966)	Reduced cerebral glucose uptake by cerebral arterio-sclerosis and diabetic encephalopathy—markedly increased by glucose insulin infusion

cross the barriers surrounding the central nervous system, be it the blood brain barrier or the cerebrospinal fluid blood barrier In other studies where ^{131}I insulin was injected intravenously into rats or human beings, no radioactivity was found in brain substance of the rat or in spinal fluid of man within the first 2 hours of injection (8, 19, 25), the conclusions were similar to those of Chowers et al, *i e*, that insulin was unable to cross these barriers From a physico-chemical point of view, however, it is unlikely that insulin with a molecular weight of 12,000 or more should be able to make this crossing in detectable

C Sp nal flu d stud es

Prepara on	Parame er s ud ed	In es ga ors	Commen
Insul n injected intracisternally (dogs)	Spinal flu d glucose decrease Blood glucose decrease Decrease greater in sp nal flu d than in blood	Cho ers et al (1961 1966)	In vagotom zed dogs s m lar decrease in sp nal flu d glucose but now ins gn f cant decrease in *blood glucose*
Sp nal flu d (crude) (homo)	Rat ep d dymal fat pad techn que no insul n l ke act ity	Mahon et al (1962) Schrader & We nges (1962) Deckert Lyngsoe & Rafaelsen (1966)	
Sp nal flu d (conc) (homo)	Rat ep d dymal fat pad techn que now demonstrable insul n l ke act vity	Deckert Lyngsoe & Rafael en (1966)	
Sp nal flu d (crude & conc) (homo)	Immuno-assay Insul n act ty 10 % of blood values	Deckert Lyngsoe & Rafaelsen (1966)	
Intravenous inject on of ^{131}I insul n (homo)	No rad oact ity demonstrable in sp nal flu d	Elgee et al (1954) Mahon et al (1962)	

amounts within a few hours This does le that insulin ultimately can barriers in amounts sufficient n important action on central stem structures but the prob it be elucidated by means of ly labeled insulin with a bio- *f life of half an hour after in* injection Insulin like activity h been demonstrated in hu rospinal fluid (7) It was muno-assay that crude spi tained some 10 per cent of a tivity of plasma from the t hereas parallel determi l that the epididymal fat ju as unable to detect these t of nsulin like activity, thus confirming negative results obtain ed by other authors (25 38) After con centration of these spinal fluid samples insulin like activity was measurable both with immuno assay and with epididymal fat pad technique Calculation of trans fer rates of insulin from blood to brain *and cerebrospinal fluid* awaits the avail ability of insulin truly labeled with ^{14}C or ^{35}S in the amino acid structures of the molecule

Chowers et al suggested parasympa thetic brain centers as a possible site of action for a direct effect of insulin on the central nervous system This may only be one of many Goodner and Freinkel (15) have demonstrated insu lin effects on anterior pituitary tissues

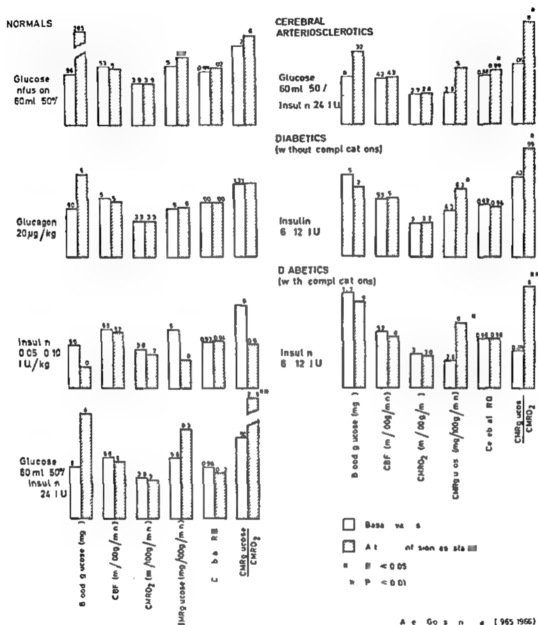
from rat and calf, both after *in vitro* and *in vivo* administration of this hormone Glucose uptake was increased by 25 per cent when anterior pituitary tissue from intact animals was incubated with insulin, and conversion of glucose to carbon dioxide and incorporation in to lipid was also increased Glucose uptake of pituitary tissue from rats rendered diabetic by alloxanization or pancreatectomy was reduced, and it could be normalized by insulin injection into the rats before sacrifice The results are in many aspects similar to results obtained with isolated rat spinal cord (29, 30), and they may be aspects of general metabolic properties of central nervous tissue It is at any rate very interesting that direct insulin effects have been demonstrated on tissues so centrally placed in endocrine homeostasis One might even speculate that insulin reaches the pituitary tissue faster than other parts of the central nervous system, due to the special properties of the hypophyseal vascular bed

In passing it may be mentioned that Levi Montalcini and co-workers (24) have demonstrated insulin action *in vitro* on growth of isolated mouse sympathetic and sensory spinal ganglia Even if these ganglia do not belong to the central nervous system, it is a note worthy finding, especially in connection with the hypothesis by Chowers and co-workers of insulin action on parasympathetic structures The number of insulin sensitive tissues and structures grows fast, soon it will be more exclusive to be insulin-insensitive!

Two groups of researchers have recently presented evidence for a direct effect of insulin on brain glucose uptake in man (4, 16, 17) Both groups have used brain blood flow determinations according to the principles of Kety and Schmidt (21) as a frame of reference in order to ascertain that changes in glucose uptake were not due to changes in cerebral blood flow Butterfield and co-workers had in earlier investigations demonstrated an insulin sensitive threshold for glucose uptake of tissues of the forearm and they have now combined these studies with parallel studies of brain metabolism Their studies showed a similar and insulin sensitive threshold for glucose uptake by brain tissue, but the threshold in brain was lower than in the forearm, and it responded more slowly to insulin The experimental design in these experiments does not compensate for insulin hypoglycemia, and the demonstration of the insulin sensitive glucose threshold in brain is therefore more indirect, but its characteriza tion as being lower and more slowly reacting to insulin may explain some of the previous failures to demonstrate direct action of insulin on brain glucose uptake in man

The most conclusive evidence to date of a direct effect of insulin on the human brain has been presented by Gottstein and co-workers (16, 17) These investigators have studied patients without any symptom or sign of brain disorder, patients with cerebrovascular diseases, and diabetic patients without and with diabetic complications In all their investigations, basal values for cerebral blood flow (CBF), cerebral oxygen uptake ($CMRO_2$) and cerebral glucose uptake (CMRglucose) were first obtained

BRAIN BLOOD FLOW AND METABOLISM IN NORMALS ARTER OSCLEROTICS AND DIABETICS



25–35 minutes later during the same investigations renewed determinations were made after intravenous infusion of glucose glucagon insulin or glucose plus insulin Some of their results are shown in Figure 1

It is seen that the basal values are in good agreement with the results obtain

ed by other workers Glucose was administered intravenously at a constant rate of 1 5 ml/min of a 50 per cent solution to a final volume of 60 ml The intravenous infusion of glucose led to a considerable increase of blood glucose values, but brain flow, oxygen uptake and cerebral vascular resistance were essentially unachanged Cerebral glucose uptake was slightly increased, but statistical analysis showed that the increase was not significant Each group of patients studied comprised from 6 to 12 individuals Intravenous administration of glucagon caused very small changes in the values for brain blood flow and metabolism under consideration here Insulin administered intravenously in a dose to reduce blood glucose values to 50 per cent caused a reduction in brain glucose uptake by a similar percentage, but brain oxygen uptake was nearly unchanged, indicating combustion of other metabolites besides glucose under such circumstances

The situation where insulin is introduced alone is unsuited to determine the direct effect of insulin on the brain, as the glucose offered to the brain by the arterial blood is markedly reduced, due to the action of the very same insulin on other organ systems It is only by the combined infusion of glucose and insulin that light is shed on this problem It is seen from the figure that the combined intravenous infusion of glucose and insulin—in spite of a smaller increase in blood glucose levels compared to glucose infusion alone—leads to a 50 per cent increase of brain glucose uptake As the cerebral oxygen uptake was unchanged, as was cerebral respiratory quotient, it is evident that the extra glucose taken up was not oxidized Its probable fate will be discussed later

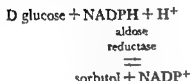
The results obtained by Gottstein and co-workers in patients with cerebrovascular diseases are also remarkable Basal values in the patients showed a 20 per cent reduction in CBF, a 25 per cent reduction in CMRO , and a 50 per cent reduction in CMRglucose, all compared to basal values in patients without cerebrovascular disorders Intravenous infusion of glucose plus insulin did not influence CBF or CMRO , whereas CMR -glucose rose to near normal values This increase was statistically very significant, as were increases in cerebral RQ and the quotient CMRglucose/CMRO• Also in diabetic patients the German group found reductions in basal values of CMRglucose which were markedly influenced by insulin infusion The reduction of basal CMRglucose was much more pronounced in diabetics with diabetic long term complications than in patients without such complications, as was the effect of intravenous insulin administration

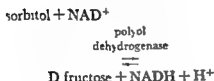
The above-mentioned findings by Gottstein and co-workers represent a major advance in the demonstration of direct action of insulin on human brain Of particular interest is the dissociation of glucose and oxygen uptake of brain after insulin infusion and also in basal uptake in patients with cerebral vascular disorders, both diabetic and non-diabetic, as it is here assumed that the disturbances demonstrated in the diabetic patients with complications are at least partly due to vascular lesions of brain vessels, *i e*, a diabetic encephalopathy

(36) In the diabetic patients more oxygen is taken up by the brain than is necessary for the oxidative consumption of the glucose taken up, after glucose-insulin infusion more glucose is taken up in normals, and after insulin infusion in diabetics, than can be accounted for by the oxygen taken up during the same period When the glucose uptake is small, the brain must turn to other substances to satisfy its most urgent metabolic needs, and on the other hand it must be assumed that the excess glucose taken up under the influence of insulin is to a great extent quickly converted to other substances This might be amino acids, lipid precursors or glycogen Several investigators have demonstrated in recent years that glucose very quickly enters the amino-acid pool in brain Vrba et al (39) found after intravenous injection of radioactive glucose into rats that already 30 minutes later 75 per cent of the radioactive glucose taken up by brain had entered the amino acid pool It is thus probable that a major part of the extra glucose taken up by brain after glucose insulin infusion enters the amino acid pool and that part of it may be used for protein synthesis It may also be supposed that in diabetic patients with complications, where glucose uptake is insufficient to cover oxidative metabolic needs amino acids in the brain are metabolized to cover the gap In the patients with cerebrovascular lesions of a non-diabetic nature similar events may take place, but here a certain breakdown of brain lipids is possible, as basal values for cerebral RQ were found moderately decreased It is obvious that continuous use of brain amino acids or lipids for immediate metabolic needs may have the most detrimental influence on brain function and brain structure Future research may reveal to what extent the well known histopathological changes of cerebral arteriosclerosis and the more recently demonstrated histopathological changes in brain of patients with long term diabetes are to be explained along the lines mentioned here

In peripheral nerve and spinal cord, metabolic derangements have been demonstrated which may have implications for insulin action on brain Insulin *in vitro* increases glucose uptake of rat spinal cord (29, 30) and of rat and rabbit sciatic nerve (9, 10) In sciatic nerve, Adams and Field (1) found a depression of the activity of the acetic thiokinase enzyme system, necessary for acetylation of Co-A as the first step in the synthesis of fatty acid from acetate, to approximately 30 per cent of that found in nerves from normal animals Insulin *in vitro* could *not* restore this enzymic function Further evidence of the important role of the acetic thiokinase block in diabetic nerve was the demonstration by the same workers that if acetate labelled acetyl Co A was used as substrate, there was no impairment of incorporation of label into fatty acid in the diabetic tissue, and the capacity for *in vitro* enhancement with added insulin was regained (10) Defective mechanisms for glucose incorporation into lipid and glycogen in diabetic rat sciatic nerve and spinal cord might lead to increased metabolization via other pathways The same workers have now (11) demonstrated a four to sixfold increase in the intracellular concentrations of

the sorbitol pathway and of free intracellular glucose in the sciatic nerves and spinal cord of alloxan diabetic rats The sorbitol pathway converts D glucose to D fructose without phosphorylation or requirement for ATP





The accumulation of the products of the sorbitol pathway may indicate that sorbitol cannot easily leave nervous tissues and that the capacity to metabolize fructose is limited As a consequence, the accumulation may exert a harmful effect by osmosis or otherwise It is noteworthy that whereas the relative increase of products of the sorbitol pathway is similar in rat sciatic nerve and in rat spinal cord, the absolute values are from 2 to 10 times lower in spinal cord than in sciatic nerve If it is assumed that the values in brain are similar to those in spinal cord (or even lower), a hypothesis may be advanced to explain some clinical and pathological findings The more pronounced accumulation of metabolites like those of the sorbitol pathway in *peripheral* nerve may lead to lesions of a metabolic, non vascular type relatively early in the course of diabetic illness These lesions may be partly reversible if the metabolic pattern in the nerves is restored by therapy or by means still unknown In the central nervous system, on the other hand, these metabolic derangements may be less harmful, and the quantities of waste-products piling up are much smaller Only later in the course of diabetes, when vascular changes become prominent also in the brain, the central nervous system will start to suffer severely due to the reduction in glucose uptake and the intermittent or constant wear and tear on the amino acid pool and brain proteins to satisfy urgent metabolic needs Thus, with our present knowledge, diabetic encephalopathy is likely to be linked to vascular changes

Insulin has for many years been used in the treatment of the major psychoses and its effectiveness in many cases has vaguely been explained as due to the induced hypoglycemic coma This can now be re-evaluated in the light of direct insulin action on the brain and especially on cerebral protein metabolism Insulin is a hormone, and it is reasonable to belive that many other hormones exert direct effect on brain metabolism and function It has been demonstrated that thyroxin influences oxidative processes in brain mitochondria and many other demonstrations will probably follow, e g, with steroid hormones

The elucidation of insulin and other hormone effects on brain will have theoretical and practical consequences in many fields of medicine

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OBSERVATIONS ON THE INFLUENCE OF GLUCOSE UPON SUBCUTANEOUS ADIPOSE TISSUE BLOOD FLOW

by

F Quaade, O Andree Larsen N A Lassen and
S Levin Nielsen

The fact that depot fat constitutes an organ with a lively metabolic activity has been well established for more than a decade, but so far quantitative studies of adipose tissue metabolism *in vivo* have been rendered difficult by the lack of a practical method for determining the perfusion rate

As described in a previous publication (2), the blood flow through human subcutaneous adipose tissue (FBF) can be measured by the local clearance technique using radioactive xenon as the indicator

Method

100 μC Xe^{133} dissolved in 0 1 ml sterile isotonic saline was injected slowly through a very fine needle into the middle of the adipose tissue layer of the abdomen, a little below and lateral to the umbilicus To avoid the artifact of the injection trauma, recording of the Xe^{133} clearance was not started until about one hour after the injection The disappearance rate of the isotope was registered with two NaI(Tl) crystals (5 cm in diameter) placed over the depot in the horizontal and vertical plane respectively In this way movements of the patient leading to an erroneous clearance curve could easily be detected The counts were registered on a conventional scaler with 100 sec intervals during every second minute for the next 1–3 hours

The calculations of the FBF-values are based on the assumptions that the area injected is homogenous with respect to blood perfusion and inert gas solubility, and that the diffusion equilibrium between fatty tissue and capillary blood is maintained

The theoretical and experimental reasons for making these assumptions, and the mathematical foundation of the calculation of FBF have been described in detail previously (1, 2) In the initial study comprising 69 examinations in 55 adult human subjects, the fasting FBF values averaged 2 63 ml/100 g min, with a standard deviation of 1 72

The normal fasting FBF values having

thus been established, the present study concerned the possible influence upon this parameter of an excess of glucose administered either intravenously or perorally

The patients studied ranged in age from 20 to 79 years and were in varying states of nutrition, from — 17 to + 50 per cent overweight (3) Apart form a mild obesity in some patients, none suffered from metabolic or circulatory diseases

The investigations took place in the morning, and the patients were fasting About one hour after injection of the xenon depot, a peripheral vein was punctured and isotonic saline was infused with a rate of 0 5 ml per min The fasting xenon clearance was recorded during 40–60 min, whereafter the saline was exchanged with a glucose solution of either 10 or 20 per cent In this way the discomfort due to the venipuncture could not influence the flow registration The xenon clearance was measured during the following 1–2 hours, and in this period capillary blood samples were taken for blood sugar analysis The peroral glucose was given in the course of a few minutes as a 50 per cent solution

Results

Our first series comprised seven subjects (table I), who were given 1000 ml 10 per cent glucose, i e 400 calories, intravenously in the course of 15 minutes As will be seen from the table, the response was extremely variable In two patients FBF remained unchanged, while in five the clearance rate increased within a few minutes after the start of the infusion, and FBF values from 46 to 600 per cent above the fasting values were reached

When the infused glucose calories were doubled, i e 1000 ml 20 per cent glucose in the course of 15 minutes (table II), the entire group of 14 subjects responded with an increase in blood flow, ranging from 111 to 900 per cent (fig 1)

In order to check the possiblity that simple plasma volume expansion might be responsible for these findings, 500 ml 10 per cent Rheomacrodex® in isotonic saline was infused in three subjects (ta-

TABLE I

Glucose 400 calories intravenously during 15 minutes

Case no	Age years	Height Weight index*) %	Fasting FBF) ml 100 g min	After glucose FBF) ml 100 g min	Difference FBF) %
53	20	−17	2 10	5 30	+152
14	23	− 2	2 91	5 46	+ 88
15	23	− 2	8 63	12 65	+ 46
17	23	− 2	5 45	5 45	0
18	23	− 9	7 48	7 48	[illegible]
22	49	+14	0 63	1 21	+ 92
29	33	+21	0 50	3 50	+600

*) According to Natvig H (1956)) Fatty tissue blood flow

TABLE II

Glucose 800 calories intravenously during 15 minutes

Case no	Age years	Height Weight index *) %	Fasting FBF **) ml 100 g min	After glucose FBF **) ml 100 g min	Difference FBF **) %
5	41	+ 4	1 15	11 50	+900
6	42	+14	4 25	10 93	+157
32	48	+15	1 78	4 49	+152
33	46	+21	1 00	3 30	+230
34	44	+18	0 90	6 00	+567
35	53	+45	7 50	15 81	+111
36	37	+27	1 72	7 07	+311
37	29	+23	2 01	9 89	+392
38	56	+ 9	1 61	6 21	+285
39	21	−10	5 00	17 74	+255
40	45	+ 5	0 98	5 81	+493
41	32	+47	0 75	5 29	+605
42	53	+ 7	1 32	2 82	+114
47	60	+11	3 22	23 89	+642

*) According to Natvig H (1956) **) Fatty tissue blood flow

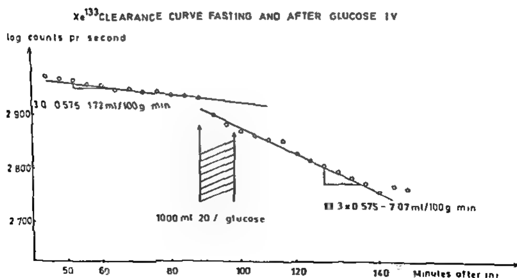


Fig 1

Xe133 clearance curve from a 42 year old normal fasting man showing the effect of an intravenous infusion of 1000 ml 20 per cent glucose during about 15 minutes

TABLE III

Rheomacrodex® 500 ml 10 % intravenously during 15 minutes

Case no	Age years	Height Weight index) %	Fasting FBF) ml 100 g min	After Rheomacrodex FBF) ml 100 g min	Difference FBF) %
7	42	+34	1 84	1 84	0
8	57	+50	1 10	1 10	0
[illegible]	53	+48	1 15	1 15	0

) According to Natvig H (1956) *) Fatty tissue blood flow

TABLE IV

Glucose oral intake

Case no	Age years	Height Weight index) %	Glucose calories	Fasting FBF) ml 100 g min	After glucose FBF) ml 100 g min	Difference FBF) %	Maximum blood sugar level mg%
37	59	+16	400	2 70	2 70	0	226
39	37	+21	400	3 20	1 50	−53	144
42	60	+13	400	3 80	1 60	−58	214
38	59	+21	800	0 90	0 90	0	227
40	61	−20	800	11 20	4 40	−61	234
41	37	+21	800	5 00	2 20	−56	182
80	42	+33	800	1 86	1 15	−38	–

) According to Natvig H (1956)) Fatty tissue blood flow

TABLE V

Glucose 800 calories intravenously during 60 minutes

Case no	Age years	Height Weight index) %	Fasting FBF) ml 100 g min	After glucose FBF) ml 100 g min	Difference FBF*) %	Maximum blood sugar level mg%
43	62	+43	2 17	0 48	−78	720
44	58	− 1	6 87	4 43	−36	950
46	74	+10	3 96	0 95	−76	269

) According to Natvig H (1956)) Fatty tissue blood flow

TABLE VI

Mannitol 500 ml 25 % intravenously during 15 minutes

Case no	Age years	Height Weight index) %	Fasting FBF) ml 100 g min	After Mannitol FBF) ml 100 g min	Difference FBF) %
77	67	+ [illegible]	7 46	31 80	326
78	79	−15	3 29	12 71	286
79	57	+13	1 87	3 74	100
[illegible]	59	+ 5	0 48	1 73	260

) According to Natvig H (1956)) Fatty tissue blood flow

ble III) No change in FBF was seen in these experiments

However, when glucose was administered by mouth, either 400 or 800 calories (table IV), none of the seven subjects thus treated responded with an increase in xenon clearance in two subjects FBF was unaltered, while in four there was even a decrease of 53 to 61 per cent

The possibility thus presented itself again that the observations in the infusion experiments were after all due to the unphysiological nature of the procedure, especially the rapidity of the infusion Infusion of 1000 ml 20 per cent glucose (800 calories) during one hour (table V) in three subjects was followed by a decrease in FBF, the values falling from 36 to 78 per cent of the fasting value

The fact that the effect on FBF of intravenous glucose seemed to depend upon the infusion rate made it necessary to test the likely possibility of a simple hemodynamic cause This time a solution comparable in osmotic effect to the glucose infusions was used 500 ml 25 per cent Mannitol solutions was infused intravenously during 15 minutes in four patients In all cases the infusion of this osmotically active substance was followed by a marked increase in FBF, starting about the end of the infusion and lasting 30–40 minutes The increase ranged from 100 to 326 per cent (table VI) of the fasting values

Discussion

The two major metabolic functions of adipose tissue are 1) the assimilation of carbohydrates and lipids for triglyceride synthesis and storage, and 2) the mobilization of lipid as free fatty acids *A priori*, it did not seem unlikely that either of these activities might be reflected in adipose tissue blood flow measurements In preliminary experiments, where liquid meals of varying composition were given to patients, we did in fact register FBF increases, and the present studies were planned so as to avoid the possible influence of an activated gastrointestinal tract, to eliminate the specific dynamic action of proteins, and to observe the effect of metabolizable carbohydrate alone The demonstration in normal persons of FBF variations under these conditions would offer further scope for investigation, especially as regards the reaction to hormones (insulin, glucagon, norepinephrine), and the response in diabetes and various types of human obesity

Out data indicate that the lipogenetic process which is known to be accelerated in the presence of insulin and surplus of available glucose is not accompanied by an accelerated fatty tissue blood flow, and the FBF increases observed after rapid intravenous glucose infusions must be explained as caused by an intravascular osmotic effect When glucose is administered under more physiological conditions, by mouth or in slow infusion, there seems to be a tendency for fatty tissue blood flow to decrease The possibility thus presents itself that free fatty acid release is a determining factor for fatty tissue blood flow, which will decrease if lipolysis is reduced in the presence of abundant glucose The data presented in this paper are neither nu-

merous nor consistent enough to decide how the observed blood flow decreases should be interpreted, and the question whether in fact variations in lipolysis are reflected in adipose tissue blood flow must await further experiments for its solution

SUMMARY

The blood flow through human subcutaneous adipose tissue of the abdomen has been measured by the local clearance technique using Xe^{133} as the indicator In a series of 55 normal subjects in various states of nutrition the blood flow averaged 2 63 ml/100 g min (SD = 1 7)

Intravenous infusion of 1000 ml 20 per cent glucose during 15 minutes resulted in an increase in fatty tissue blood flow ranging from 110 to 900 per cent of the fasting values The blood flow decreased about 50 per cent after a similar amount of glucose given either intravenously during one hour or taken by mouth The increase in flow seemed to be due to the osmotic effect of the rapidly infused glucose, as the infusion of 1000 ml 25 per cent Mannitol also provoked a pronounced increase in fatty tissue blood flow

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INSULIN

DESIRABLE AND UNDESIRABLE EFFECTS

by

Jacob E Poulsen

The introduction of insulin in 1922 must still be considered one of the most important medical advances of the century It has not only added many years to the lives of millions of diabetics, but for a multitude of people it has turned the existence of an invalid, in starvation and constant danger, into an almost normal life in the family as well as in society, where now only a few occupations are not open to diabetics

Numerically, diabetes is really a mass disease From sporadic group studies it may be estimated that about 1 per cent of humanity is affected If this is taken to apply to all populations, about 30 million people are diabetic Among somatic diseases this can only be exceeded by certain tropical diseases, such as malaria and bilharziasis, and by nutritional diseases—in the western world overnutrition and in Africa and Asia undernutrition

Only about 30–40 per cent of diabetics are dependent upon permanent insulin therapy, while the remainder need it only periodically in the event of intercurrent diseases and surgical procedures, to tide them safely over the critical episodes Thus, to all diabetics insulin is either a permanent necessity or a security After the advent of insulin, the lifetime of diabetics has been prolonged This applies particularly to children and adolescents, whose survival prior to the insulin era was 2 or 3 years, while now it is 25–30 years and still increasing

From the time of the first biological demonstration of the hypoglycaemic effect of pancreatic extract by Banting and Best in July 1921, intensive research has been going on to clarify the chemistry, physiology, and pharmacology of insulin

Most progress seems to have been made by the chemists as insulin was the first protein to be crystallized in 1926, by J J Abel, and its amino acid sequence was elucidated in 1955 by F Sanger, who also demonstrated certain species differences Lastly, insulin was also the first protein to be synthesized This has been done by three different teams within the past 3 years (H Zahn of Aachen, P Katsoyannis of U S A , and Wong Hu of Peking) Chemists have displayed quite a special interest in

insulin, which had represented a special challenge to them by virtue of its great medical significance Its small molecular size and its easily demonstrable biological properties have no doubt contributed to this great interest

The physiological effects of insulin still remain unelucidated, but hypotheses on its effect at the molecular level have not been wanting

In addition to the hypoglycaemic effect, the diabetic organism was soon found to exhibit other phenomena following administration of insulin, viz decrease or cessation of ketosis, increase in muscle and fatty tissue, normal growth of diabetic children, increased resistance to infections, and a possibility of carrying through pregnancy These observations go to show the anabolic effect of insulin, also confirmed *in vitro* in which it is possible to demonstrate the promoting action of insulin upon the formation of glycogen, fatty acid and protein from low molecular metabolites such as glucose, pyruvate, acetate and amino acids

Whether this stimulation of macromolecular synthesis is due exclusively to the easily demonstrable increased transport of the named low-molecular metabolites from the extra to the intracellular compartment, or whether this process is caused also by a direct intracellular activity of insulin, is still an open question Recent physiological theories have of course included also the microarchitecture of the cells

Cori s interpretation (1946) of insulin action as a stimulation of hexokinase activity in muscles has been revived recently (1966) by S Bessmann s model of the insulin effect, in which insulin is believed to effect mechanical binding between mitochondria and the cytoplasmic hexokinase in the muscles or glucokinase in the liver This sets up a system in which glucose is phosphorylated, converting ATP into ADP ADP is necessary for oxidative phosphorylation, and in diabetes the metabolic anomalies may be explained as deficient regeneration of ADP This view may also explain why insulin is not needed for the glucose metabolism in the brain, as in the cells of the brain the hexokinase is bound to the large intracellular particles

Clinical Use

In the early insulin era there were numerous problems concerning the production of suited injection preparations for daily use This was the first time that the pharmaceutical industry was faced with the problem of developing an injection medicine which was to be administered by the patients themselves over long periods

The first preparations were in the form of tablets or powder which had to be dissolved before use Partly because of this procedure and partly because the pancreatic extract also contained substances other than insulin, there would often be local reactions, in some cases abscess formation, at the sites of injection

In 1924, insulin was dispensed as a sterile solution in ampoules, and this reduced the main local complaints following the injections However, a few days after the commencement of insulin therapy, reddening and swelling at the injection sites were not uncommon, but

these complaints subsided with continued use

As the insulins were rid of unwanted associated proteins, the effect of the injections became more rapid and more short lasting Especially in children and adolescents, it was apparent that the effect of the evening injection was too brief to keep the morning blood sugar at the desired level and to keep the patients free of ketonuria in the morning

It was an important advance, therefore, when H C Hagedorn, Norman Jensen, N B Krarup, and I Wodstrup, *in 1936 (9)*, gave *their report* on the first long acting insulin preparation This made it possible

(1) to reduce the daily dose of insulin,
(2) to reduce the glycosyria and ketonuria, and
(3) to reduce the hypoglycaemic attacks

N B Krarup, in his thesis from 1936 (11), gave a thorough analysis of the clinical effect of protamine insulin During the intervening 30 years there has of course been progress in the production of insulins, but strangely enough protamine insulin still has its prominent place among the insulin preparations of to-day

From 1946, protamine insulin was sold as a crystalline product whose crystals have a shape quite different from insulin crystals Protamine [redacted] used only in 10 per cent of the weight of the insulin for producing the isophanic crystalline protamine insulin It has been elucidated that protamine is split by plasmin whereupon insulin is released from the injected depot Zinc inhibits this enzymatic process and furthermore makes the insulin less soluble This has been utilized in the protamine zinc insulins (8), in zinc insulin suspensions, and in zinc insulin crystals as in the Lente series (10)

It [redacted] a drawback of all insulins that they do not release insulin from the depots according to the requirements of the body at all times, as does the normally functioning pancreas, but that the insulin [redacted] released from the depot in a manner characteristic of each preparation It is important, therefore, to know how to utilize the possibilities afforded by *the different preparations for individ*ualized insulin therapy

It now appears to be generally accepted that it is a great advantage to be able to use mixtures of regular insulin and long acting insulin in cases of juvenile diabetes of some duration The insulin sensitivity is relatively low during the early morning hours, presumably related to the diurnal variation in adrenocortical function, which shows maximum plasma levels of cortisol in the early morning

The use of insulin in psychiatry [redacted] outside my scope, but in spite of recent years enormous, almost alarming, development in the field of psychopharmacology, insulin treatment is still in use in various mental diseases

Undesirable Effects of Insulin

Insulin induced hypoglycaemia and its symptoms have been known from the earliest days of insulin In spite of the development in insulin preparations, the physicians' familiarity with the hypoglycaemic syndrome and widespread ed

ucation of the insulin treated diabetic by doctors, popular articles and the efforts of diabetic associations, hypoglycaemic attacks still constitute a problem in the treatment of diabetes

The symptom complex of hypoglycaemia stems mainly from the central nervous system The explanation is that the metabolism of the CNS depends almost exclusively *on glucose as a source* of energy, but it has only a small store of glycogen The nervous system has a great demand for energy and a great oxygen consumption During hypoglycaemia the oxygen saturation of the blood is satisfactory, but nevertheless the symptom complex is similar to anoxia airing to the reduced possibility of procuring glucose to burn

The first signs are due to the release of adrenaline and manifest themselves as sweating, an increased pulse rate, slightly elevated blood pressure, pupillary dilatation, hunger, and trembling These signs are so striking that most diabetics can arrest the further development of the hypoglycaemia by taking carbohydrate Unfortunately, a number of diabetics do not notice these initial symptoms, and therefore cannot take measures in time The initial signs are most marked when the fall of blood sugar is rapid, and therefore they may be less noticeable when slow acting insulins are used Incidentally, the symptom complex manifests itself as blurring of cortical function in the form of lacking concentration and change of mood, often into a contrary attitude, also to therapeutic suggestions from the surroundings The patients may develop mental confusion, and in the event of more profound disturbances somnolence, loss of consciousness, grimacing, clonic spasms and deep coma, during which the Babinski sign is positive The symptom complex often develops rapidly in connection with unusual physical activity, as a rule some hours after the last major meal Full blown hypoglycaemic coma calls for glucose intravenously, possibly *injection of glucagon In some cases we* have furnished the mothers of diabetic children with glucagon which they can give their children, if they have difficulty in getting medical aid quickly

Hypoglycaemia with a blurred cortical function is of course a risk to the patient as well as to others in our highly developed technical society, not least in traffic Nevertheless, it seems to be uncommon in the Scandinavian countries for diabetics to be involved in major traffic accidents, no doubt because diabetic drivers are carefully instructed and because they know that they may lose their driving licence in the event of a traffic accident caused by a hypoglycaemic state

Most insulin reactions subside without permanent complaints, in most cases resulting only in headache for a few hours In extremely rare cases they may cause permanent damage to the central nervous system, e g hemiplegia or aphasia Death in hypoglycaemia following therapeutic doses is rare Insulin has been used for suicidal purposes, but then usually in enormous doses

The pathological changes in the brain following fatal hypoglycaemia are reminiscent of the changes seen after fatal anoxia, with scattered ischaemic haemorrhages

In countries where different strengths or concentrations of the insulins (20–80 units/ml) are used, mistaking one preparation for another has not infrequently caused hypoglycaemia It seems reasonable, therefore, to allow only one strength, viz 40 units per ml A fairly unheeded cause of hypoglycaemia is the change from a long used site of injection to a site not used previously We have occasionally seen hypoglycaemia following massage of tissues injected with insulin for some time

Hypoglycaemia results in the release of the insulin antagonistic hormones For more than 30 years it has been realized that adrenaline is released This causes a release of glucocorticoids, and in recent years it has also been realized that hypoglycaemia gives rise to the secretion of somatotrophin

This hormonal counter-regulation causes partly glucogenolysis from the liver and muscles and partly peripheral insulin resistance Teleologically, all this may be interpreted as the possibilities that the organism has for procuring the glucose needed for the normal function of the nervous system

It was one of the advantages of the slow-acting insulins that hypoglycaemic attacks could be reduced and possibly prevented, as demonstrated by N B Krarup in his thesis It is less known that hypoglycaemia may also increase the ketosis in a patient who is already slightly ketotic (Fig 2) On the other hand, this ketonaemic reaction does not occur

Fig 1

Influence of hypoglycemia on the blood sugar and sugar excretion

if the blood levels of ketone have been elevated during the hours prior to hypoglycaemia (Fig 3)

Owing to this phenomenon, the breath of unconscious hypoglycaemic patients may smell of acetone, and this may lead to the hasty diagnosis of a hyperglycaemic ketoacidotic state, with therapeutic consequences which may prove fateful to the patient

The unphysiological route of adminis tering insulin has in some cases caused

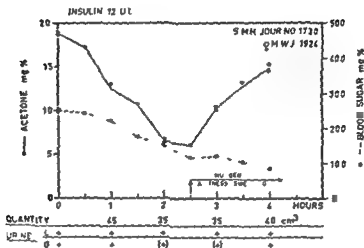


Fig 3

Blood s ... nia following insulin During hypoglycemia there is an increase in the ketonemia

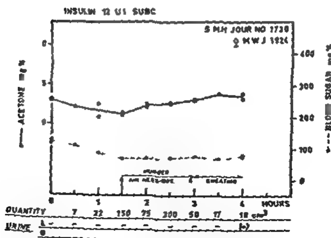


Fig 3

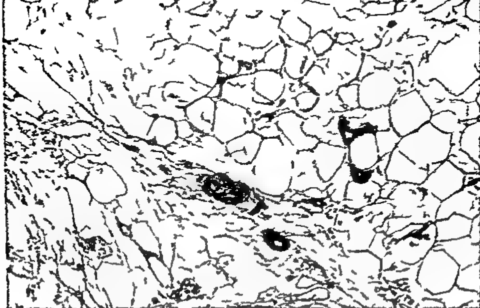
The same patient as in Fig 2 after the cessation of the ketosis A very slight increase in the ketonemia is now observed



Fig 4
NSH 11781/65 ♀ born 1937 Diabetes 25 years
Injection pads after injections in the same area during many years



Fig 5
NSH 11059/65 ♀ born 1950 Diabetes 1964
Lipodystrophia following insulin injections in one year



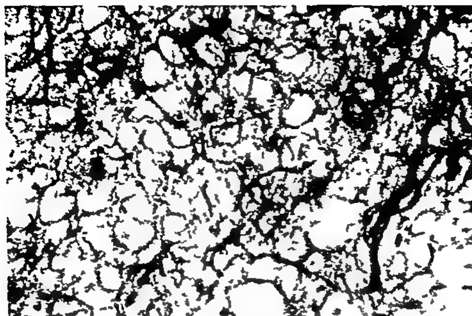
F g 6

\SH 108? /65 ♂ III years diabetes 1 year Insul n treatment for one year
B opsy of in ul n injected subcutaneous area 200 × PAS + HA
The cells vary con derabl n size S reaks of connect e tissue elements and inf ltra n
cells are observed Augmentation of vessel wall th ckness (A Werner NSH)



F g 7

Same as F g 6 Closer v ew Detail 400 ×
Cel ular nf l rat on cons s s of eos noph le and neutropl e leucocytes
l mphocytes and his ocytes
The thickened appearance of ves el walls and fa cell memb anes is obv ous
A Werne NSH



F g 8

From the same pat ent as Fig

Subcutaneous t ssue PAS + HA 215 ×

Incubat on th iod ne 125 labelled nsul n and sub equent autorad ograph c expos re
B nd ng of I 125 nsul n to fat cell membranes and vessel walls th a d st nct increase
of PAS pos e substance (A Werner ASH)



F g 9

Same preparat on as F g 6

B nd ng of fluoresce n sot ocyanate-conjugated nsul n to essel alls
and fat cell membranes

Segmentary d str but on of the fluorescence could be no ed n the vessels 215 ×

local abnormalities at the sites of injection, especially in patients who do not change the sites of injection sufficiently often In some instances this may result in thickening of the skin and subcutaneous tissue, at times assuming a monstrous degree, as seen in Fig 4 In the case, depicted, subcutaneous tissue to an amount of 400 g and 250 g was removed at the patient s wish, to normalize the contours of the thighs The excised tissue showed pronounced changes, lobulated fatty tissue with connective tissue strands and minor haemorrhages of varying age

In other cases there may be wasting of fatty tissue, i e lipodystrophy, in the injected areas or nearby, as shown in Fig 5 This latter phenomenon of lipodystrophy is most marked in children and women, while it is rare in men, perhaps because of their usually thinner layer of subcutaneous fat

Both of these local effects of the insulin injections naturally give rise to cosmetic complaints, but it has also been found that after injection into the thickened areas, the effect of insulin is incalculable and erratic In recent years we have removed cutaneous and subcutaneous biopsies from the injection sites in several cases, and the specimens have shown considerable tissue changes (12)

The biopsies from the thickened areas exhibit fat cells of very unequal and partly very large size There are strands of connective tissue and also cellular infiltration by lymphocytes, macrophages, plasma cells and eosinophilic leukocytes The walls of the smaller vessels appear to be thickened with proliferation of endothelial cells and abnormal increase of PAS positive material in the vessel wall (Fig 6 and 7)

In the lipodystrophic areas there is almost total loss of fatty tissue and degenerative changes of the connective tissue Studies have also been carried out after treatment of the tissue section with I^{131}-labelled insulin (1) in a concentration of 150 units per ml for 30 min, rinsing drying, and autoradiography (Fig 8) Similar studies have been performed by fluorescein-conjugated insulin (14) in the named concentration and examination in ultraviolet light (Fig 9)

These studies showed marked binding of the two labelled insulin preparations, a binding which could be prevented by preceding treatment with non labelled insulin Biopsies from outside the injected areas showed less marked binding of insulin to the vessels and tissue membranes

Using labelled insulin, it has been possible since 1956 to demonstrate the occurrence of insulin antibodies in the blood of insulin-treated patients (2) Since then, extensive studies have been performed in this field (7) It is generally accepted that insulin antibodies are not demonstrable by immunological methods in patients who have not previously been treated with insulin, *and it is also* accepted that insulin antibodies invariably arise after parenteral insulin therapy for some time

The cause has not been definitely elucidated There are several possibilities

(1) that the antigenicity is caused by lacking species specificity,

(2) that in the course of extraction the insulin alters chemically, acquiring antigenic properties during the procedure,

(3) that owing to its manner of administration, viz introduction into subcutaneous tissue with traumatization of the tissue, and in a concentration up to a million times higher than the plasma level of insulin, the insulin sets up an immunological reaction directed at an otherwise non antigenic molecule

Re 1 It has proved possible to produce antibody also by the use of species specific insulin (4, 14)

Re 2 In the immunological technique used, the natural insulin in plasma reacts like the extracted insulin

Re 3 At present, this view seems to me to be the most likely one, as there are cellular signs of delayed immune reactions, specific binding of the antigen to vessels and cell membranes, as well as antibodies in the plasma In many respects this reaction is reminiscent of an autoimmune disease in which the organism also forms antibodies to a molecule which is not antigenic under normal conditions

In my opinion, the possibility cannot be ruled out that the chronic allergic reaction induced by the insulin therapy is a contributory cause of the late diabetic complications in insulin-treated patients On the other hand, it has been definitely demonstrated that such complications also develop in diabetics who are not treated with insulin If this latter group should represent an autoallergic condition, it is of a 'submethodological" degree or it is due to another antigen than that in the insulin treated patients, who can naturally be imagined to be subject to two chronic allergic processes

SUMMARY

Insulin therapy has prolonged the lives of patients suffering from diabetes with a tendency to ketosis In these patients the duration of diabetes has been increased at least ten times after the advent of insulin therapy Moreover, insulin therapy is of great importance as a temporary measure during intercurrent diseases and in the event of surgical procedures on diabetics who are not on permanent insulin therapy

The chemistry of insulin has been elucidated in all essentials The amino acid sequence is known, and insulin can now be synthesized The physiological action of insulin still remains unelucidated

The clinical use of insulin has been characterized by the development of highly purified insulins absorbed at different rates from the injected depot

Untoward effects of insulin are, in part, hypoglycaemia with its complex of acute symptoms and signs, mainly from the central nervous system, and in part local changes at the sites of injection Such changes consists in microscopic abnormalities of the fatty tissue, delayed immune reactions, small haemorrhages, formation of connective tissue, and in rare cases lipodystrophy Furthermore, the blood of insulin treated patients contains antibodies directed at insulin This formation of antibodies is presumably due to the unphysiological way in which insulin is administered

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POSTPRANDIAL BLOOD SUGAR RISE IN DIABETICS

M Jersild

Determinations of blood sugar level and of sugar in the urine are indispensable in evaluating the treatment in diabetics It is desirable to obtain values approaching normal as far as possible There has always been a disagreement, however, about where to fix the limit for satisfactory regulation During ambulatory treatment it is generally not possible to arrange more than a single blood sugar determination per day In this case the fasting blood sugar is usually preferred as guidance for treatment, perhaps together with one or two blood sugar determinations before the main meals Occasionally, only determinations of sugar in the urine are used

It is often rather surprising to find a high sugar excretion in a patient where the above mentioned blood sugar values are quite satisfactory Consequently, one might suppose that the patient has a low renal threshold for sugar if the postprandial rise in blood sugar is not borne in mind

The blood sugar rise 1 hour after breakfast has been found to be remarkably high in diabetics—also when compared with the rise after the other main meals which contain relatively more carbohydrates

The postprandial blood sugar values after all meals were recorded in 16 diabetics during hospitalisation 11 blood sugar determinations were performed in each patient in 24 hours Of the 16 patients, 6 were treated with one insulin injection a day, 9 patients with 2 injections, and 1 patient did not get any insulin The average age of the patients was 32 years The average duration of the diabetes was 14 years The results are seen in *table I* the largest postprandial blood sugar rise was found af

TABLE I

Postprandial blood sugar rise in 16 patients and average carbohydrate content in meals

	Time of day	Av rise of blood s (mg%)	A carboh content of m als (g)
1h after breakfast	9	108	37
1h after lunch	12^{30}	31	59
1h after tea	16	30	23
1h after dinner	18^{30}	70	58
1h after evening	22	28	13

ter breakfast (108 mg per cent), although this meal had a lower carbohydrate content than lunch and dinner The carbohydrate content of breakfast in Denmark represents on an average 20 per cent of the total carbohydrate intake

In order to determine the rise in the blood sugar after breakfast (at 8 o' clock) in a larger number af diabetics —with or without insulin treatment— the postprandial values were recorded one hour after breakfast in 312 consecutively hospitalized patients during a period of 6-7 days 2013 blood sugar determinations, measured by the Hagedorn Jensen method, made one hour after breakfast, were used in these investigations

The age distribution is seen in *fig 1* The majority of the patients are younger than 40 years of age

The duration of the diabetes is on an average between 5 and 10 years

The rise in blood sugar from 8 to 9 o'clock in the morning is on an average 90 mg per cent The fall from 9 o'clock to 11 30 (before lunch) is on an average 104 mg per cent

In *fig 2* the patients are classified according to the postprandial blood sugar levels one hour after breakfast

One third of the patients (group I) had

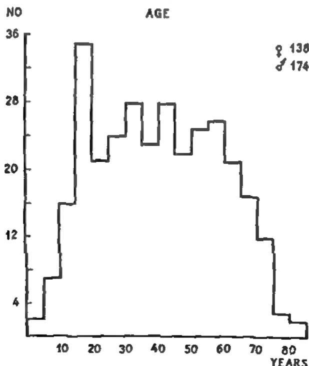


Fig 1

Age and sex distribution in 312 diabetics

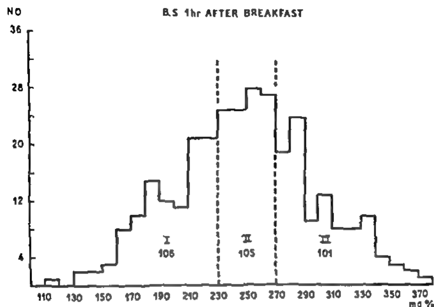


Fig 2

Postprandial blood sugar values 1h after breakfast
Group I 106 pts with values 110–230 mg%
Group II 105 pts with values 231–270 mg%
Group III 101 pts with values >270 mg%



Fig 3

Age distribution in the 3 groups

postprandial blood sugar values
from 110 to 230 mg per cent
Another third (group II)
from 231 to 270 mg per cent
The last third (group III)
from 271 to 370 mg per cent

In the following, the question will be examined whether these 3 groups show any correlation to other clinical manifestations of the disease

Fig 3 shows the age distribution in the above mentioned 3 groups The conclusion is that there is no correlation between the postprandial blood sugar rise and the age of the patients It might be supposed that the young diabetics with unstable blood sugar were to be found mainly in group III, but this is not the case

Fig 4 shows that the postprandial blood sugar rise is not correlated to the duration of the diabetes The average duration in group I is 13 years, in group II 12 years, and in group III 15 years

However, a significant correlation is *found between the postprandial blood* sugar rise and the age of the patients at the onset of the diabetes (*table II*) the diabetics with juvenile onset were found more frequently in group III (84 per cent) with the highest postprandial blood sugar values than in group I (67 per cent) with the lowest values, and the maturity onset diabetics were found more frequently in group I (33 per cent) than in group III (16 per cent)

The carbohydrate content of the breakfast is not correlated to the blood sugar rise (*table III*) In group I, II, and III the average carbohydrate content of the breakfast is practically the

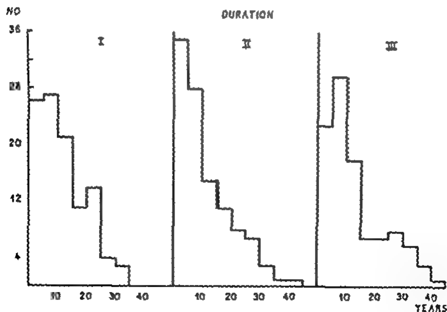


Fig 4
Duration of diabetes in the 3 groups

same (37 g) As expected, the sugar in the urine (*table IV*) during 24 hours is higher in group III (32 g) than in group I (11 g)

The obese patients are found more frequently in group I (*table V*), these patients generally belonging to the maturity onset, non insulin treated, stable diabetics

The frequency of retinopathy and nephropathy in the 3 groups is seen in *table VI* practically the same incidence of retinopathy (average 57 per cent) was found in the three groups, whereas

TABLE II

Age at onset in the 3 groups

Group	I	II	III
<40 years per cent of pts	67	72	84
<40 years per cent of pts	33	28	16
Total no of pts	106	105	101

TABLE III

Carbohydrate in breakfast

Group	I	II	III
Av grams	36	37	38

TABLE IV

Urinary sugar in the 3 groups

Group	I	II	III
No of pts	103	104	97
Urinary sugar av grams/24 h	11	19	32

TABLE V

Overweight in the 3 groups

Group	I	II	III
≥10 % overweight per cent of patients	32	25	10

TABLE VI

Long term diabetic manifestations in the 3 groups

Group	I	II	III
% retinopathy	57	58	56
% nephropathy	25	15	9

nephropathy occurred more frequently in group I than in group II and III It is difficult to give an entirely acceptable explanation of this phenomenon, but the relative prevalence of obese patients with maturity onset diabetes in group I should be considered

The treatment in the three different groups has also been investigated (*table VII*) In the insulin treated patients, the average insulin dose was almost the same in all three groups One daily injection is given more frequently (63 per cent) in group I than in group III (40 per cent) The contrary was the case in the two injection therapy 14 of the patients did not receive any insulin treatment Of these, 12 were found in group I and 2 in group II This means—as suspected—that the non insulin treated patients with a stable blood sugar also had the smallest postprandial blood sugar rise

The highest postprandial blood sugar values are found in the unstable diabetics This has also been demonstrated by evaluating the other blood sugar values of the day by means of the M value (5)

Discussion

The pronounced postprandial blood sugar rise after breakfast has been recorded previously by the author (2, 3)

By means of continuous blood sugar determinations, Chaptal, Guillaumot and Morel (1) in 1964 demonstrated this considerable blood sugar rise in children in spite of a breakfast relatively poor in carbohydrates Sindoni (6), in 104 diabetics, found an average blood sugar rise of 100 mg per cent 2 hours after the breakfast

Mirouze et al (4) demonstrated the marked oscillations of the blood sugar during the day and night by means of continuous blood sugar determinations The highest values were found 1/2 hour to 2 hours after the main meals, and in the labile diabetics they were only slightly influenced by the type of insulin used or the time when the injection was given

An explanation of the pronounced postprandial blood sugar rise after breakfast may be the higher level of blood corticosteroids during the morning than in the evening A Somogyi effect following a nightly hypoglycemia should not be overlooked

Conclusion

The postprandial blood sugar rise is highest after breakfast, in spite of this meal containing relatively little carbohydrate

The higher content of corticosteroids

TABLE VII

Insulin dose and No of insulin injections in the 3 groups

Group	I	II	III
No of pts	94	103	101
Insulin av dose i u	31	34	36
1 inj per cent of pts	63	54	40
2 inj per cent of pts	37	46	60

in the blood during the morning hours may be the cause of this phenomenon In two-thirds of the diabetics, the postprandial blood sugar values after breakfast increased to more than 230 mg per cent

The highest postprandial blood sugar rise was found in diabetics with juvenile onset of the disease This blood sugar rise could not be avoided even when the patients were given 2 insulin injections per day

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EXTRAPANCREATIC AND INTRAPANCREATIC ACTION OF ANTIDIABETIC SULPHONYLUREAS A REVIEW

by

Joop Madsen

Since the early work of Loubatieres, an enormous literature has appeared on the mechanism of action of the antidiabetic sulphonylureas and on their clinical application Thus, a bibliography only on tolbutamide published in 1965 (129) contained more than 1700 numbers The commonly accepted conclusion of this work has been that the hypoglycemic sulphonylureas lower the blood sugar by stimulating the insulin secretion of the β-cells in the islets of Langerhans The purpose of the present paper is to call attention to a number of findings that cannot be explained by a pancreatic locus of action and to discuss the hypoglycemic effect of the sulphonylureas on the background of these experiments

The chemical composition of the antidiabetic sulphonylureas is shown in fig 1 Apparently there is no principal difference in the mechanism of action of these compounds so that they will be treated as one group in the following Extensive reviews are available of the literature up to 1960–61 on the mechanism of action of the sulphonylureas (34 38, 81)

β-cell stimulation by sulphonylureas

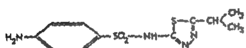
As early as 1944, Loubatieres (71) demonstrated that p-aminobenzenesulfamidothiodiazol (2254RP) caused hypoglycemia in dogs, and that this effect depended on the presence of pancreas or part of it in the animal Loubatieres reported in the same paper that one-tenth of a hypoglycemic dose would elicit a fall in blood glucose level, if injected into the artery leading to the pancreas, and he found an increase in liver glycogen after 2254RP, as after insulin He concluded that the compound lowered the blood sugar by stimulating the insulin secretion of the β-cells in the islets of Langerhans

Following the appearance of carbutamide and tolbutamide in the midfifties, a great amount of work was done on the mechanism of their hypoglycemic action, largely confirming the early work of Loubatieres on 2254RP

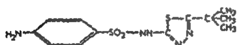
It was shown that the sulphonylureas were inactive in the pancreatectomized, non insulin treated dog (14, 23, 41, 55, 61, 86, 94), cat (46), rabbit (58), rat (27), toad (55) and man (33)

It was further demonstrated that the responsiveness of alloxan diabetic ani mals to sulphonylureas was inversely re lated to the severity of the diabetes so that severely diabetic animals would not respond while animals with a milder diabetes would react to sulphonylureas with a hypoglycemic response though less so than normal animals (1 10 15 30 72 82 92) Similarly the reaction of animals with a metahypophysary dia betes would be reduced according to the severity of the diabetic state (68 74 90) It was concluded that the hypo- glycemic sulphonylureas depended on the presence of functioning β cells for their ability to lower the blood sugar

This conclusion was supported by sta tistical comparison of the sulphonylurea responsiveness in populations of diabet ics differing with respect to age sex age at the beginning of their diabetes dura tion of the disease and their insulin re quirements It was found that the pa tients who responded to sulphonylurea treatment where those whose diabetes developed at a mature age who had on ly been sick for a short number of years



p-a inobenzen-sulp onamid iso ropyl t iodia ol
(4 M)



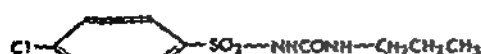
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(22)



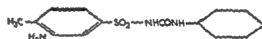
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(C rbut mide)



1 (4 met l n e o) 2 lu a
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N1-(4 lorbenzen l o ro yl r a
(lor ro ide



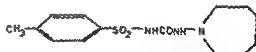
N1 (3-amino-4 m tylbenzensulphonyl) -cyclohexyl
ur a (Metaher mide)



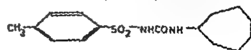
N1(4 a et lben ensulpho yl) N cyclohexylurea
(Aceto exa i e)



1(4 me yl thiobe zensulphonyl) cyclohexylur
thiohexamide



1(4 m tylbe zens lp onyl) hex hydroaz pinylur
(Tola mid)



1(4 metylbenzensulphonyl) ycloheptylur a
(Cycl hep olamide)

F g 1

An d abet c sulphonam des and sulphonylureas

and whose insulin requirements were modest The same correlation was found between the parameters mentioned above and the amount of extractable insulin recovered from diabetic patients at autopsies (17, 138, 139) Thus, a similarity was evident between the responsiveness to sulphonylureas and the availability of insulin in the pancreas

Histologic examination of the islets of Langerhans after administration of sulphonylureas showed a degranulation of the β-cells and characteristic changes in their nuclei A survey of 42 investigations on this subject is given in (81) Since the β-cell granule consists of insulin or insulin precursors, the degranulation indicates that sulphonylureas trigger a release of preformed insulin from the pancreas This interpretation is supported by measurements of extractable insulin in pancreas of calves (103), dogs (113) and rats (37) before and at various time intervals after sulphonylurea administration

An increase in plasma insulin like activity after sulphonylurea administration has been demonstrated by the rat hemidiaphragm (3, 11, 53, 101, 123, 130) and rat epidedymal fat pad (102) techniques as well as by immunological methods (140) and *in vivo* assays (4, 44) Perfused isolated pancreas (84) as well as incubated slices of pancreas (20) release more insulin under the influence of sulphonylureas than in the control period

Finally, a number of insulin effects have been recorded *in vivo* as well as *in vitro* after the administration of sulphonylureas Among these is a reduction of the glucose output of the liver (9, 28, 59, 60, 109, 119, 121), which can also be seen after slow, intraportal infusion of small amounts of insulin (12, 28, 59, 79) An increased extrahepatic glucose uptake has been more difficult to demonstrate It has been done, however, but Butterfield et al (21) Searle et al (120, 122) have shown that both tolbutamide and insulin increase $C^{14}O_2$ production from a trace dose of C^{14}-glucose in normal men as well as in sulphonylurea responsive diabetics

The findings listed above constitute the main body of evidence on which the conclusion is based that the antidiabetic sulphonylureas exert their hypoglycemic effect by stimulation of the insulin secretion from the β-cells of the pancreas Many investigators have failed to reproduce insulin effects by the administration of sulphonylureas This difficulty, however, is explained by the different effects of insulin given intravenously as a single injection and insulin secreted slowly into the portal circulation

Extrapancreatic action of sulphonylureas

A number of findings however, cannot be explained by a pancreatic action of the antidiabetic sulphonylureas Many investigators have produced blood sugar falls by the administration of sulphonylureas to pancreatectomized (13, 56, 61, 114, 116 119, 128) or severely alloxandiabetic (2 114, 116) animals treated by small amounts of insulin Chronic administration of sulphonylureas to pancreatectomized dogs will reduce their insulin requirements (24, 61, 111, 115, 118, 124, 136) Kurtz et al (64) found that tolbutamide injected intravenously

into dogs within 10 minutes after total pancreatectomy, would elicit a blood sugar fall, while no significant effect could be detected if the injection was given 1–2 hours after pancreatectomy. Obviously the presence of insulin and not the presence of an intact pancreas is the condition for the hypoglycemic effect of the sulphonylureas.

This extrapancreatic "insulin potentiating" effect of sulphonylureas is not only seen when the compounds are given to insulin-treated animals without functioning β cells, but also if the insulin sensitivity of such animals is investigated in acute experiments with and without sulphonylurea given simultaneously (25, 69, 75, 93, 94). This insulin potentiating action has also been demonstrated in diabetics who were insensitive to sulphonylureas given alone (69, 89). Increased insulin sensitivity has been reported during chronic sulphonylurea treatment of unresponsive diabetic patients (126) and pancreatectomized or alloxan diabetic dogs (112). Experiments by Caren & Corbo (25) on pancreatectomized dogs indicate that the demonstration of this sulphonylurea-effect depends on the dosage of insulin. Insulin must be administered in a suitably small dose, otherwise no difference can be shown between animals receiving insulin and animals receiving insulin + sulphonylurea. This fact may account for the numerous futile attempts that have been made to demonstrate this insulin sulphonylurea synergism in pancreatectomized humans (29, 33, 45, 83), dogs (41, 86, 94) and cats (46) as well as in alloxan-diabetic rabbits (125) and rats (36, 67, 88, 110).

Experiments by Schambye & Tarding (119) using the glucose-C^{14} dilution method showed that the blood sugar fall after tolbutamide given intravenously to insulin treated pancreatectomized dogs was caused by an inhibition of the glucose output by the liver. Another hepatic, insulin potentiating action of carbutamide has been demonstrated by Lamprecht & Trautschold (66). Though without effect when given alone, carbutamide reinforces the effect of small doses of insulin, so that—like much higher doses—they normalize triosephosphate metabolism in livers of hunger-diabetic rats. Insulin potentiating effects on the liver have also been reported by Colwell (26).

The "insulin potentiating effect", however, can not only be localized to the liver. This is apparent from experiments of Houssay et al. (57), Madsen (80) and Creutzfeldt et al. (32). In eviscerated dogs, cats and rats, they have shown that falls in blood sugar can be produced if tolbutamide is injected into animals whose blood sugar has been maintained constant or increasing by mean of a constant rate glucose infusion, and to which minute amounts of insulin have been administered (fig. 2).

In the cat experiments it could be calculated that the glucose utilization in eviscerated animals given insulin 0.01 unit/kg i. v. + tolbutamide 100 mg/kg i. v. exceeded the utilization in eviscerated animals given only insulin, by 25 per cent ± SEM 6.5 per cent. In hepatectomized cats with intact pancreas, in which the blood sugar was maintained constant or slightly increasing by means of a constant rate glucose infusion, tol

butamide 100 mg/kg i v increased glucose utilization by 38 per cent ± SEM 6 8 per cent The difference between the tolbutamide effect on glucose utilization in hepatectomized cats with an intact pancreas and in eviscerated cats without a pancreas, but treated with minute doses of insulin, is not significant ($P < 0.1$) Thus, it cannot be excluded that the tolbutamide effect in the hepatectomized cat is entirely extrapancreatic

In the experiments of Houssay et al as in those of Madsen, it was found that the amount of insulin given was critical for the tolbutamide effect Thus, an increase in insulin dosage from 0 01 to 0 02 units/kg markedly reduced the tolbutamide effect in eviscerated cats

A number of authors (95, 99, 104, 105) have found that sulphonylureas increase the glucose uptake of isolated skeletal muscle from normal rats At the same time oxygen uptake (95, 104) and $C^{14}O_2$ production from labelled glucose (12, 47, 104) are increased *An in vitro* effect of tolbutamide has also been described (70) in fat pads from animals with intact pancreas Rafaelsen & Lundbæk have observed an increased glucose uptake by diaphragms from alloxan diabetic rats (106) The possibility cannot be excluded, however, that minute amounts of insulin remained in their preparations

Summarizing it may be concluded that the hypoglycemic sulphonylureas can indeed lower the blood sugar level by increasing extrahepatic glucose utilization by a mechanism that requires the presence of small amounts of insulin, but not of the pancreas In the following this effect will be called the insulin-potentiating effect of the sulphonylureas

What can the mechanism of this effect be? Mirsky et al (91) and Tamiay & Kizima (127) have advanced the hypothesis that the sulphonylureas lower the blood sugar by inhibition of enzymatic

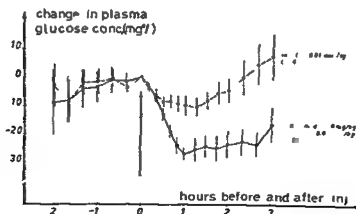


Fig 2

Insulin potentiating effect of tolbutamide in eviscerated glucose infused cats Injection of insulin or insulin + tolbutamide was performed at the arrow Average values are marked ± SEM The differences between the two animal groups are statistically significant ($P < 0.05$) from 40 minutes after the injection (From 81)

insulin degradation However, since the usual hypoglycemic concentrations of sulphonylureas do not inhibit "insulinase (16, 133), and since other compounds have a powerful insulinase inhibiting effect without being hypoglycemic (137), this theory must be regarded as obsolete Nor can a decreased production or effect of anti insulinary hormones be the explanation, since neither removal of the pituitary, thyroid, adrenal or sex glands diminishes the hypoglycemic effect of the sulphonylureas (73) On the contrary this is increased after adrenalectomy (10, 23, 55, 108, 134) It has been suggested that insulin might be of importance for the penetration of sulphonylureas into the cells (52), and that an especially active sulphonylurea insulin complex might be formed (31, 69), but neither of these theories has been supported by any experimental evidence Experiments designed to demonstrate such a sulphonylurea-insulin complex were negative (23, 81)

It must be considered whether interference with other insulin antagonists could explain the insulin potentiating effect of the sulphonylureas Two of these antagonists, however, depend on the presence of an intact pituitary gland, namely Bornstein's β-lipoprotein factor (19) and Vallance Owen s synalbumin antagonist (131, 132) Since the sulphonylureas are fully active in the absence of the pituitary, these factors can be left out of consideration The same is the case for Field & Stetten's α globulin (39) which has only been demonstrated during diabetic acidosis Lowy et al (76) have described an insulin antagonist in the serum albumin This factor resemble Vallance Owen's synalbumin, except in that it does not depend on the presence of pituitary Whether the sulphonylureas interfere with this factor is unknown

Inhibition of insulin inactivation by plasma components

A number of findings strongly suggest that insulin is present in normal as well as diabetic plasma in different forms, some of which are biologically and/or immunologically inactive

While added insulin from man or ox moves with albumin and α globulin in electrophoresis (5, 107), plasma insulin like activity (ILA) can be detected in all electrophoretic fractions, mainly in the albumin-α_1-globulin fraction and in the β-γ globulin fraction (77) These findings suggest that part of the insulin is bound to some other protein

The availability of anti-insulin sera has permitted the distinction between ILA that can be inhibited by anti insulin and ILA that will not be inhibited by anti insulin Another distinction between insulin like activities in plasma can be obtained by simultaneous ILA determinations using rat diaphragms and rat adipose tissue as test objects The rat diaphragms will give lower ILA's than the adipose tissue, and the ILA determined on muscle will all be anti insulin suppressible, while the higher values obtained with the adipose tissue have a suppressible and a non-suppressible component In addition to these fractions, serum contains 'hidden ILA" (43, 78), ILA that cannot be detected

biologically or immunologically in untreated serum

Part of the non suppressible and hidden ILA can be converted to suppressible ILA by acid ethanol extraction (117) or by dialysis (78) Thus, it can be regarded as well established that at least part of the non suppressible ILA is really insulin

Antoniades (5, 7) has reported that a Dowex 50 cation exchange resin will retain part of the ILA as measured by the adipose tissue method, while nearly all ILA as measured with rat diaphragm will run through ("free insulin) After elution of the retained ILA, it can be shown to be non suppressible by anti-insulin and to move with the β, γ globulins in electrophoresis ('bound insulin") "Bound insulin was shown to be a complex of insulin and a protein with isoelectric point about pH 10 By various treatments, 'free insulin' can be split from this complex Antoniades has also found "bound insulin" in extracts from pancreas (8)

That non-suppressible insulin consists of particles with a higher molecular weight than suppressible insulin is also evident from the work of Samaan et al (117), who found that immunologically suppressible insulin could ultrafiltrate, while non suppressible ILA could not

An extensive review on the state of insulin in plasma is outside the scope of this paper For such a review, including a discussion of the possible identity of insulin fractions described by different authors, the reader is referred to the recent study by Lyngsoe (78) However, it may be concluded that a significant part of plasma insulin is present in high weight molecular form, possibly bound to plasma proteins, protected from anti insulin, inactive towards muscle preparations but in part active on adipose tissue In the following, this ILA will be called 'bound insulin' With this expression the author does not particularly mean the "bound insulin' described by Antoniades, unless this is specifically stated

Looking for a mechanism to explain the extrapancreatic, extrahepatic, insulin-dependent hypoglycemic action of the sulphonylureas, it would be natural to consider an effect on the "bound insulin" If the sulphonylureas are able to release free insulin from "bound insulin , they would increase the concentration of insulin effective on skeletal muscle

This theory has first been put forward by Krahl (63) and it has been mentioned as a possibility by Linke (69) Krahl supports the hypothesis by the finding that sulphonylureas increase the glucose uptake by isolated diaphragms, if they are incubated in normal plasma, but not if they are incubated in buffer Details of these experiments have not been published Lacy (65) reported that tolbutamide added in vitro at a concentration of 100 mg per cent increased the ILA (fat pad technique) of sera from 8 of 12 insulin treated diabetic patients, and from 6 of 13 non diabetic patients This tolbutamide concentration is 3 times the concentration used in the cat experiments reported on p 112 and 12–15 times therapeutic concentrations Hasselblatt (50) has reported that tolbutamide added at a concentration of 20 mg per cent to serum from normal, thyroxin

treated rats will significantly increase the ILA (measured on rat diaphragm) This increase was found to depend on the dietary state of the animal, being most pronounced 6–10 hours after feeding Estimating ILA in normal guinea pig sera by net gas exchange by rat adipose tissue, Otto & Korner (98) found that the ILA was increased by addition of tolbutamide to the serum If exogenous insulin was added, no tolbutamide effect could be detected Thus, a parallel is seen to the experiments in eviscerated (57, 80) or pancreatectomized (25) animals, where the tolbutamide effect depends on the presence of insulin in very small amounts Several unsuccessful attempts have been made to repeat Hasselblatts experiments in other laboratories (35, 62, 100)

In this connection, another finding of Hasselblatt et al (51) is of interest, namely that tolbutamide inhibits the binding of bromsulphalein to serum proteins

Antoniades et al (6) have examined the content of "free" and "bound insulin" in the blood of non diabetic and non insulin dependent diabetics before and after intravenous injection of tolbutamide In 7 out of 13 non diabetic cases they observed a pronounced increase in the concentration of "free" and a fall in the concentration of "bound insulin" after the tolbutamide injection In 4 cases the effect was mild, and in two no effect of tolbutamide was seen Of 9 diabetic patients, 3 showed a mild the rest a pronounced effect While the increase in "free" insulin may be due to an increased secretion of insulin from the pancreas the fall in "bound insulin" suggests a dissociation or an otherwise increased utilization of "bound insulin" under the influence of tolbutamide

Gundersen et al (49) have found that the presence of heparin in serum will cause a dissociation of "bound insulin" Using this method, Gundersen examined sera from non-diabetics and non-insulin requiring diabetic patients before and after oral tolbutamide tests, finding no appreciable effect on the dissociation of "bound" insulin (48)

An antagonistic effect on the binding of insulin to anti insulin sera has been reported by Hasselblatt (50) Lacy (65) found that addition of tolbutamide to sera from guinea pigs immunized with crystalline ox insulin increased the ILA of the serum However, tolbutamide would not split ox insulin antibody complexes Similarly, Otto and Korner (98) found that tolbutamide would not prevent the binding of added insulin to insulin antibodies, although tolbutamide would increase the ILA of sera from immunized animals, to which insulin had been added From these experiments they concluded that the sulphonylureas do not liberate bound exogenous insulin, but somehow activate endogenous, inactive insulin in the serum

When labelled insulin was injected into diabetic patients, the activity disappeared faster from plasma if the patients were treated with tolbutamide, than if they were not (18) Since tolbutamide treatment did not accelerate the elimination of labelled metabolites, it is unlikely that tolbutamide increased insulin degradation However, the results may support the concept of an inhibited

binding of insulin to plasma components

Many cases have been reported of lowered insulin requirements in insulin resistant diabetics during sulphonylurea treatment (54, 85, 87, 96, 97) Such reductions amounting to several hundreds of insulin units daily can hardly be explained by an increased insulin secretion However, they may be explained by an inhibited binding of insulin to plasma proteins (antibodies?) During tolbutamide treatment of a resistant patient, Friedlander & Bryant (40) found that the insulin binding effect of serum was markedly reduced Hasselblatt (50) found that tolbutamide added in vitro at a concentration of 22 mg per cent would significantly decrease the insulin binding effect of serum from an insulin resistant patient Full neutralization of the insulin binding effect was reached at a concentration of 70 mg per cent (10 times therapeutic concentrations)

Finally, a paper by Wallenfels et al (135) should be mentioned here They found that addition of carbutamide prevents the aggregation of insulin molecules that otherwise takes place in the presence of Zn Based on these findings the authors suggest that carbutamide and other sulphonylureas may be able to loosen bindings of insulin to other proteins, i e serum proteins They consider that sulphonylureas break up insulin aggregates in the β-cells thereby rendering insulin soluble and able to leave the cells

DISCUSSION

The findings mentioned in the previous section are not unequivocal, and many of them have been difficult to reproduce They do however point towards the fact that sulphonylureas somehow activate or potentiate inactive insulin present in plasma On the background of our increasing knowledge of 'bound' insulin in plasma, it seems a reasonably well-founded hypothesis that the sulphonylureas promote a liberation of "bound" to "free" insulin It has more experimental support than any other theory put forward to explain the insulin dependent effect of the sulphonylureas in pancreatectomized and eviscerated animals

Reviewing the experimental evidence on which the β-cell stimulation theory is based (p 109–111), it will be seen that most of these findings can as well be explained by insulin potentiation This applies to the increased ILA in pancreatic veins as well as in other parts of the circulation, to the observation of insulin effects on hepatic as well as extrahepatic metabolism and to the necessity of functioning β-cells in the patient or experimental animal The histological changes in the β-cells and particularly the measurements of extractable insulin in pancreas before and after sulphonylurea administration must however be taken as proof of an increased secretion of insulin under influence of the sulphonylureas Thus, their mechanism of action is twofold partly stimulating insulin secretion, partly activating or releasing inactive, bound insulin extrapancreatically

Little is known about the relative contribution of these two mechanisms to the hypoglycemia seen in intact humans

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THE IN VIVO REACTIONS OF THE SMALL BLOOD VESSELS TO DIABETES MELLITUS

by

Jorn Ditzel

Diabetes mellitus of long standing is characterized by clinical signs which can be attributed to a generalized degeneration of the small blood vessels. The histological changes in these lesions share certain similarities in various organs, characterized by endothelial proliferation, hyalinization and periodic acid Schiff (PA-S) positivity. For many years the structural changes were thought to be limited to the renal glomerular and retinal capillaries, but it is now recognized that the lesions are much more widely distributed. The designation "diabetic microangiopathy" has gained acceptance for this distinctive systemic disease of the microvasculature.

Much has been learned from the morbid anatomy of this condition. However, if one accepts the development of diabetic microangiopathy as a slow chain of event from the earliest evidence of diabetes, further elucidation of this process is most likely to come from dynamic observations of the micro-vascular system at an early stage of the disease. Such detailed studies of the *in vivo* changes of the terminal blood vessels in diabetic subjects have recently been reported. Based particularly on biomicroscopic observations of the bulbar conjunctiva, it has been demonstrated that severe functional alterations of the vascular bed may be observed from the onset of diabetes and even in close relatives to diabetics (prediabetics?). These reversible changes appear to be precursors of the degenerative vascular changes, and observations indicate that similar reversible changes in other tissues participate in the mechanism of the development of the long term diabetic syndrome (21).

It is the purpose of this paper to review our present day knowledge of the *in vivo* reactions of the small blood vessels observed in particular detail in the conjunctiva of diabetic patients.

EARLY STUDIES OF THE CONJUNCTIVAL VESSELS

Historical aspect. The conjunctival vessels in diabetics have been under scrutiny for many years. However, because many of the studies were performed with unacceptable methods, and the results presented in the form of vague statements without supporting objective clinical data, the significance of most of these investigations is difficult to assess. Hirsch-

berg (25) in 1890 was the first to refer to the conjunctival vessels in diabetics by his brief mentioning that conjunctival haemorrhages were more liable to occur in diabetic than in non diabetic persons This observation has not been confirmed in later studies (43, 19) Streiff (40), Zeller (46), and Lohlein (35) all commented upon the presence of changes of a degenerative nature in the conjunctival vessels of patients with renal diseases, diabetes mellitus, rheumatoid arthritis, arteriosclerosis and anemia Koby (30) mentioned that considerable dilatation of the conjunctival veins was frequently observed in diabetics

Do microaneurysms identical to those of the retina occur in the conjunctiva? It was, however, the discovery of the microaneurysms in the small retinal venous channels as an important feature of diabetic retinopathy (4) which first gave impetus to the study of the conjunctival vessels of diabetics Friedenwald (22), using the corneal microscope, studied the conjunctival vessels *in vivo* in 60 patients with diabetic retinopathy Only one "microaneurysm" was found in this series, and Friedenwald mentioned that similar aneurysm-like dilatations occasionally could be seen in non-diabetic subjects In contrast to this result McCulloch & Pashby (36) claimed the occurrence of a high incidence of "aneurysms" analogous to the retinal microaneurysms in the conjunctival vessels of diabetics In studying with the slit-lamp the conjunctival vessels of 100 elderly diabetics with and without diabetic retinopathy and 50 hospitalized non diabetic patients, they recognized the incidence of "aneurysms" to be 55 per cent among the diabetics compared with 14 per cent among the non-diabetics A higher incidence of "aneurysms" occurred in the diabetics with retinopathy (62 per cent) than in the diabetics with no retinopathy (48 per cent) The authors suggested that the "aneurysms" in the vessels of the conjunctiva were part of a generalized vascular disease, diabetes mellitus, affecting the retina, kidney glomeruli, and particularly the islets of Langerhans Weinstein & Forgacs (45) briefly mentioned, without supporting data, that they were able to confirm the finding of McCullosh & Pashby However, their presentation of microphotographs showed marked variation in shape of the conjunctival "aneurysms" over a short period of time, and thus presented a contrasting feature to the structural changes of the microaneurysms in the retina Cook (8), using a slit lamp, studied *in vivo* the conjunctival vessel of 250 diabetics with and without retinopathy and 250 non-diabetic subjects of comparable ages He found a slightly increased incidence of conjunctival 'aneurysms' in the diabetic group (34 8 per cent) as compared with the control group (29 per cent) The difference between the two was not significant and the greater incidence in the diabetic subjects was ascribed to the greater incidence of hypertension in this group However, the following considerations detract from the value of this investigation The term 'aneurysm' was not applied to capillary microaneurysms, but to fusiform, saccular and irregular dila

tations of unspecified small vessels Cook's illustrations showed that these changes were observed in the pericorneal vessels These vessels of the conjunctiva are a unique type, because they have to provide nutrition for the avascular cornea and are situated in that part of the conjunctiva which is constantly exposed to air and occasional trauma One objection valid for all the studies of the conjunctival vessels of diabetics mentioned is that the subjects used were mainly elderly diabetics in whom changes in the vascular bed due to aging and hypertension are superimposed upon the possible diabetic vascular changes It is well recognized that aneurysm like dilatations in the venules are manifestations of the aging process even in healthy subjects (42, 29) In reinvestigating the question as to whether capillary microaneurysms similar to those in the retina occur in the conjunctival vessels of diabetics, Ditzel & Duckers (15) examined 70 diabetic children and 70 healthy children In this study a microaneurysm was defined as a globular capillary sacculation with visible afferent and efferent vessels Not a single case of such microaneurysms was found in either the diabetic or the non-diabetic group In addition, in a study of 150 normotensive diabetics and 90 healthy subjects, evenly divided as to sex and with ages varying from 16 to 75 years, no specific conjunctival lesions analogous to those occurring in the diabetic retina could be found (11) Lestradet & Labram (33), by biomicroscopy, observed conjunctival aneurysms in only one case among one hundred young diabetics (1 per cent) and in 4 cases among 205 control subjects (2 per cent) Davis & Landau (9) investigated the conjunctival vessels in 551 persons, consisting of 75 patients with diabetes, 145 with hypertension, 100 with arteriosclerosis and 161 with different chronic medical diseases and 65 healthy subjects Aneurysms as defined by the work by Ditzel & Duckers (15) were found in 8 per cent among the diabetics and in 1 per cent among the non-diabetics, frequencies which do not differ significantly from each other They noted also like Weinstein & Forgacs and Cook that the "aneurysms" changed in shape, and in this way they differed from the retinal aneurysms Chazan et al (7) examined the bulbar conjunctiva for "micropools" in mothers who had given birth to babies weighing more than 4 kilos, 8 to 10 years prior to the study They found a high incidence of micropools in younger women, but the micropools were found as commonly in mothers with normal glucose tolerance test as in mothers with diabetes

In summary There exists some controversy as to the presence and incidence of aneurysm like dilatation in the conjunctiva of diabetic subjects The reason for this discrepancy undoubtedly stems from a lack of definition of what is considered an "aneurysm", in that some investigators under the designation "aneurysms" or "micropools" include the frequently occurring fusiform or saccular dilatation of small venules It appears justifiable to conclude that in diabetics there are no changes in the conjunctiva analogous with the retinal microaneurysms Since Ashton (1) pointed out the apparent absence of such microaneurysms in any tissue other than the

retina, it appears that the formation of retinal microaneurysms is related in some way to local factors, such as a high cellular metabolism in combination with the special capillary morphology, arrangement and high capillary pressure in the retinal tissue (12)

THE CONJUNCTIVAL CHANGES AS PART OF THE DIABETIC MICROANGIOPATHY

Although no microaneurysms can be found in the bulbar conjunctiva, other changes of a reversible or irreversible nature have been described and may provide data applicable to the microangiopathy of diabetes

If the vascular changes in any tissue are to be accepted as a part of the diabetic microangiopathy they must satisfy the following requirements Firstly, the changes must occur considerably more frequently in a group of diabetic patients than in an otherwise comparable group of non-diabetic subjects secondly, the incidence of the changes must increase with the duration of diabetes, and thirdly their incidence in the group must increase in diabetics with retinopathy and nephropathy Do the conjunctival changes satisfy these requirements?

The individual features indicative of degenerative changes in the bulbar conjunctiva, such as arteriolar and venular irregularities, venular sacculations and hyaline infiltration occur more frequently in the diabetic than in the corresponding non-diabetic group (13 19) Besides this category of changes capillary elongation increased venular arteriolar diameter ratio (V/A ratio) and microscopic oedema preponderated among the diabetics The difference between the degree of conjunctival changes in diabetics and non-diabetics was highly significant, particularly in the younger age groups ($p < 0.01$) Lestradet & Labram (33) confirmed this result in a study of the conjunctival vessels of 100 young diabetic subjects In an analysis made on data obtained from examination of 70 diabetic children, Ditzel & Duckers (15) found a positive correlation between the degree of conjunctival change and the duration of the disease This correlation was not influenced by either the sex or the age of the children Janert & Olbert (26), using a classification of the conjunctival vascular changes similar to that used by Ditzel & Sagild (11), studied 225 diabetics When the material was subdivided into 156 patients with less than 8 years duration and 69 patients with more than 8 years duration of diabetes, they found that the incidence of more severe conjunctival changes was significantly greater in the patients with the longer duration It was further shown that a significantly positive correlation existed between the changes in the conjunctival tissue and the presence of retinopathy Ditzel (19) in a blind study' investigated the conjunctival changes in 60 younger diabetics with diabetic nephropathy or retinopathy or both, and in 35 diabetics of corresponding ages without late diabetic manifestations The conjunctival changes were significantly more often of a severe degree among the group of patients with retinopathy or nephropathy than among diabetics without these late diabetic vascular manifestations It was

demonstrated that the relation between the occurrence of conjunctival changes and nephropathy or retinopathy did not simply reflect a common dependence on the duration of diabetes

In a few cases, Funahashi & Fink (23) have also shown histologically that the conjunctival changes may be associated with PAS positive thickenings of the capillary walls similar to the histological changes of small blood vessels elsewhere in the diabetic patient Further histological studies of the conjunctival vessels in diabetes are badly needed

In summary No degenerative lesions occur in the conjunctival tissue which are specific to diabetes However, the difference between the incidence and distribution of conjunctival changes in diabetics and non-diabetics is marked, and particularly so in the younger age groups The degree of conjunctival changes is positively correlated to increasing duration of diabetes and the presence of retinopathy and/or nephropathy The conjunctival changes can therefore be considered a part of the diabetic microangiopathy

FUNCTIONAL CHANGES IN THE CONJUNCTIVAL VESSELS

Ditzel & co-workers (15, 16, 17, 18), Bech et al (5), Lestradet & Labram (33), Rees et al (37) have all observed that besides morphologic changes, reversible alterations also take place in the conjunctival vessels of diabetic persons The change most frequently observed was a reversible dilatation (loss of vascular tone) of the collecting- and post capillary venules When the general dilatation became marked, it extended into the venous part of the capillaries, and localized fusiform sacculations were formed in the collecting venules There occurred some redistribution of flow from the "true capillaries to the arteriolar-venular shunts (20) As the venules became engorged, evidence of microscopic oedema was observed (Vascular Pattern Change I) (Figure 1) Some diabetics, particularly cases with long duration of their disease or cases with nephropathy and hypertension, may demonstrate a characteristic avascular pattern (Vascular Pattern Change II) (15) In this group the arterioles are markedly constricted Most true capillaries are closed and the tissue ischemic The direct arteriolar venular communications (shunt vessels) remain open, and become a dominant feature of the vascular topography (Figure 2) The rate of blood flow is considerably decreased, including that in the arterioles, and pronounced erythrocyte aggregation is usually present The most harmful effect of the loss of venular tone is a decrease in the linear rate of blood flow through the microvasculature and pathological permeability The continuous seepage of plasma components through endothelium leads to oedema and over a prolonged time to hyaline mucoid changes in the tissue (15, 16) The most harmful consequence of the prolonged constriction of arterioles and capillaries and shunting of blood would be prolonged hypoxia and subnutrition

If the observed pathophysiological pattern deviations are to be considered a part of the diabetic microangiopathy (a *functional microangiopathy*), they must

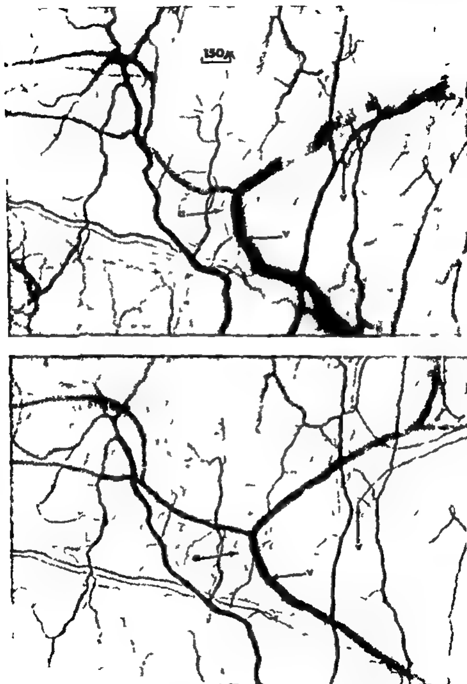


Fig 1

The reversibility of venular dilatation (v) and congestion (Vascular Pattern Change I) in a juvenile diabetic subject (Magnification approx 60 ×)

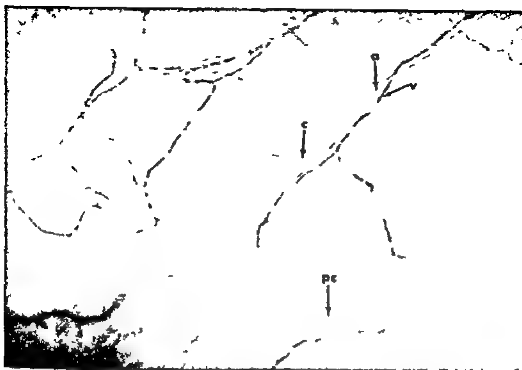


Fig 2

The conjunctival vascular bed showing Vascular Pattern Change II in a young diabetic subject Note the ischemia of the tissue (arteriole a venule v) Most of the true capillaries (c) are closed but the arteriolar venular communcations are open (pc) (Magnification approx 70 ×)

also satisfy the following requirements 1) The pattern changes must occur considerably more frequently in a group of diabetic patients than in an otherwise comparable group 2) Their incidence in the group must increase with the duration of diabetes 3) Their incidence in the group must increase in diabetics with retinopathy and nephropathy All these three requirements have been shown to be fullfilled for the pathophysiological response changes (15, 17) Figure 3 indicates the incidence of Vascular Pattern Change I in various groups of diabetic and non-diabetic subjects The venular dilatation was present in 2 per cent of the healthy children as opposed to 47 per cent of 70 diabetic children Vascular Pattern Change I could be seen even in young diabetics whose disease was of recent onset In a selected group of 75 supposedly normal children of juvenile diabetic mothers in whom an oral glucose tolerance test (GTT) was made independently of the study of the small blood vessels, venular dilatation was observed in 65 per cent of those with abnormal GTT (chemical diabetes), as opposed to 29 per cent (16 cases) of the children with normal GTT (10) Of even more interest was the finding that among the 16 cases with normal GTT, all of whom showed Vascular Pattern-Change I, 3 developed overt diabetes

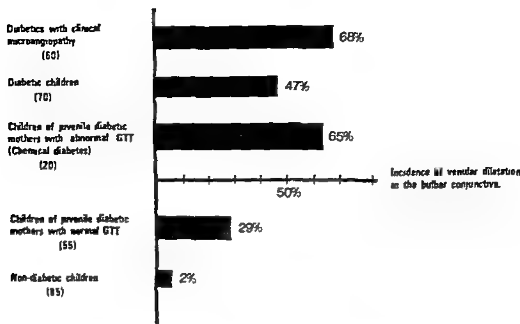


Fig 3

Incidence of venular dilatation (Vascular Pattern Change I) in the bulbar conjunctiva in different groups of individuals (see text)

and one showed a border-line diabetic GTT when reexamined 3 years later 14 More recently, Camerini-Davalos et al (6) in a study of subjects with a history of diabetes in both parents or who were identical twins of diabetic patients found that the mean widest V/A ratio in 92 per cent of these subjects prediabetics') showed values above 2 81 *which is the mean of the normals* + 2 S D Rees & co-workers (37) demonstrated the reversibility of the venular dilatation in the conjunctiva of young newly discovered diabetics after insulin and dietary treatment had started In cases of short term diabetes, fluctuations in the venular dilatation might occur over the day but the venular anomaly tended to become fixed in the majority of diabetics with a duration of 15 years or more (18) The presence of conjunctival venular dilatation in diabetics, measured by different methods, has been confirmed by Bech et al (5), Lestradet & Labram (33) and Landau & Davis (31) Ditzel & Duckers (15) demonstrated a significant association between the presence of dilated venules and microscopic oedema of the conjunctival tissue, indicating an abnormal permeability through these small vessels even in juvenile diabetics early during the disease In studying 70 unselected diabetic children, divided into two groups according to the duration of diabetes, Ditzel & Duckers (15) found that Vascular Pattern Change I occurred more frequently among those with a longer du

ration of diabetes, and the presence of abnormal vascular patterns was related significantly to the duration of diabetes ($p < 0.01$). Ditzel, Sargeant & Hadley (17) evaluated the incidence of abnormal vascular patterns in 60 young diabetics showing retinopathy and/or nephropathy and in 69 diabetics without such complications (Table 1). Abnormal vascular patterns were found significantly more frequently among the diabetics with retinopathy and/or nephropathy (83 per cent) than among diabetics without such lesions (51 per cent). In general, among the 60 young diabetics with vascular complications, those who showed Normal Vasular Pattern demonstrated only minimal vascular disease as compared to those diabetics who showed Vascular Pattern-Changes I and II. Even though the duration of diabetes was comparable in the groups under investigation, the diabetics who exhibited Normal Vascular Pattern appeared to have a much better prognosis with respect to the rate of development of their vascular disease than the young diabetics demonstrating Vascular Pattern Change II.

In summary: Severe functional vascular changes have been demonstrated in the microvasculature of the conjunctiva in diabetics, leading to an impairment and a redistribution of flow in the microcirculation. Vascular Pattern-Change I, commonly found in young diabetics, is characterized by a loss of tone of the venules and the venous part of the capillaries associated with exudation. Vascular Pattern Change II, occasionally observed in long term diabetics, is characterized by marked arteriolar constriction and capillary closure. Vascular Pattern Changes I and II are significantly related to the presence of microscopic conjunctival exudation. The incidence of Vascular Pattern-Changes I and II increased significantly with the duration of diabetes, but Vascular Pattern Change I occurred even in diabetics with a short duration of their disease. The degree of venular dilatation tended to reverse as dietary intake and insulin effect improved the metabolic disturbance. Young diabetics with long term diabetic microangiopathy showed a significantly more severe vascular pattern abnormality than those diabetics not showing retinopathy and/or nephropathy. These correlations support the supposition that the functio-

TABLE 1

Abnormal Vascular Responses in Young Diabetics With Retinopathy and/or Nephropathy and in Young Diabetics Without Such Lesions

	60 Young Diabetics With Clinical Microangiopathy		69 Young Diabetics Without Clinical Microangiopathy	
Normal Vascular Pattern	17 %		49 %	
Vascular Pattern Change I	63 %	83 %	48 %	51 %
Vascular Pattern Change II	20 %		3 %	

nal changes are a part of the diabetic microangiopathy (a *functional microangiopathy*)

COMPARISON OF MICRO VASCULAR CHANGES IN THE CONJUNCTIVA WITH THOSE IN OTHER VASCULAR BEDS

Skin As the conjunctiva and the subcutaneous tissue are anatomically and embryologically related, and their blood vessels react similarly to vasomotor drugs (38 27), it is of interest to compare the *functional, vascular changes in the conjunctiva* with those found in other surface areas of diabetics

It is well known that many diabetics have flushed faces (rubeosis) Skin colour, in general, is mainly determined by the amount of colour transmitted through the skin from the venous plexus situated in the corium Weil (44) studied the mucosa of the cheek and the capillaries of the fingernail bed in a number of diabetic patients He found in both surface areas that the venous part of the capillaries and particularly the venules in approximately 50 per cent of the diabetics were considerably dilated He contended that this was caused by a loss of tone Modern monographs on diabetes usually pay little attention to the clinical sign of rubeosis possibly because its assessment is difficult in many cases. However it should be noted that Lundbæk (34) found this phenomenon in as high as 47 per cent of 163 long-term diabetics More recently Gitelson & Wertheimer Kaplinski 24 found that rubeosis may not even be related to overt diabetes as a high incidence was found in latent diabetes A deliberate search for diabetes among 150 patients with "red faces" revealed 39 new cases, mainly of latent diabetes In another recent study Landau & Davis (31) found changes characterized by a dilatation of the venous part of the capillaries in the nailbed in 49 per cent of 75 diabetics, but only in 10 per cent of 65 control subjects

Retina Since the changes in the retina are the first decisive sign of the long-term diabetic sequelae, it is of interest to examine whether microvascular alterations similar to those directly observed *in the conjunctiva occur in the retina* of patients with diabetes Due to technicalities, however, such a direct comparison is not possible The ophthalmoscope affords much less magnification than the device applied to the conjunctival microcirculation Therefore, caliber changes in the terminal arterioles, the capillaries and smaller venules cannot be disclosed in the retina Only when the changes involve the small veins can these be observed with the ophthalmoscope However, when the retinal veins are studied, it would appear that there are striking similarities between the pattern of changes occurring in the conjunctiva and in the retina Larsen (32) studied diabetics serially with the modern retina camera and reported that fullness or dilatation of the retinal veins might be observed, even from the onset of diabetes During the first years of the disease the fullness and dilatation of the retinal veins were reversible but later on venous dilatation was prone to be more permanent The reversibility of the dilatation of the retinal veins appeared to be related to the degree of regulation of the metabolic

disturbance More recently Jutte (28) carefully measured the retinal veins by the method of Lobeck and found that the retinal veins were dilated in 43 per cent of 100 juvenile diabetics The dilatation increased with the duration of diabetes during the first 10 years An increase in vein diameter occurred during periods of poor regulation, but was reversible with correction of the diabetic metabolic disturbance In many juvenile diabetics, Jutte as well as Thiel (41) observed that the dilatation extended into the small venules and "capillaries" (rubeosis retinae) Because of the reversibility of the changes in cases of short-term diabetes, Jutte considered the dilatation a functional change He suggested that the capillary venous dilatation was produced by some alteration in the metabolism of the retinal tissue and that the dilatation on the venous side of the microcirculation caused prolonged venous stasis It is well known that the formation of capillary microaneurysms is not absolutely specific to diabetes, but has been clinically and histologically demonstrated in a variety of non-diabetic clinical disorders associated with prolonged retinal venous stasis This indicates that the aneurysms may be related to factors common to many diseases which are present to a particular degree in the diabetic state (20, 2, 3) Skovborg et al (39) have reported on a comparative study of vessel diameters in the retina, measured from the negative of retinal photographs of 492 juvenile diabetic and healthy subjects The preliminary results of this large scale study indicate that ■ venous dilatation occurs prior to any other retinal change and that the increase in venous diameter ■ significantly correlated to the duration of diabetes The results thus support and extend the finding by Jutte There ■ also evidence of a functional disorder of the glomerular capillaries in young diabetics early during their disease, as indicated by increased glomerular filtration rates These studies have been surveyed in another publication (21)

CONCLUSION

Direct *in vivo* observations of the reactions of the small blood vessels in the bulbar conjunctiva, skin, and retina of diabetic patients demonstrate severe functional changes in the microvasculature early during the course of their disease and prior to any signs of the clinical microangiopathy The common features of these functional changes are a loss of normal vascular tone and increased permeability This *functional microangiopathy* is intimately related to the development of the clinical, degenerative microangiopathy It ■ therefore suggested that the functional changes are precursors of the degenerative vascular changes and participate in the mechanism of the development of the long term diabetic syndrome

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CAPILLARY DIFFUSION CAPACITY FOR SODIUM IN SKELETAL MUSCLE IN LONG-TERM JUVENILE DIABETES MELLITUS

by

Jens Trap Jensen, Joseph S Alpert, Garcia del Rio and *Niels A Lassen*

Characteristic vascular lesions have been described in a number of tissues in patients with juvenile diabetes mellitus These lesions could in many cases be correlated with the clinical signs of long-term diabetes and it appears that the vascular lesions constitute a morphological basis for the long term diabetic syndrome The most specific vascular lesion is capillary microangiopathy The initial morphological changes consist of thickening of the basement membrane and periendothelial deposition of PAS positive material Goldenberg et al (4) first described this microangiopathy in skeletal muscle capillaries This observation was confirmed by Pedersen and Olsen (16) in a material of skin and muscle biopsies They pointed out that the lesions were diffuse but arranged segmentally Electron microscopic studies of muscle capillaries (2, 5, 26) have shown the occurrence of patchy thickening of the basement membrane often with vacuolised rarefactions Although much attention has been paid to the morphological changes in diabetic microangiopathy, very little is known concerning the physiological behaviour of these thickened capillary basement membranes

Ismail et al (7) observed reduced permeability to albumin in patients with diabetes in a study of transcapillary protein diffusion Rossing (20), however, was unable to confirm this observation Decreased water filtration in diabetic pregnant women was described by Spetz (23) who measured the capillary filtration rate for water by plethysmography on the forearm

This investigation presents evidence of increased capillary permeability for the sodium ion in skeletal muscle of patients with long term juvenile diabetes This evidence was obtained using the ^{24}Na and ^{133}Xe local clearance techniques (10, 11) in patients with clinical signs of diabetic angiopathy

MATERIAL AND METHODS

Thirteen non-diabetic subjects (3 females and 10 males) ages 21–40 years

(mean 29.1 years) were studied as one control group. These subjects had neither family histories of diabetes mellitus, nor evidence of glucose in the urine, nor any symptoms of arterial disease in the lower extremities at the time of the examination.

Seven patients (3 females and 4 males) with recently-diagnosed juvenile diabetes mellitus, ages 16–25 years (mean 19.3 years) with symptoms of the disease during not more than three years (mean duration 1.9 years) were studied as a second control group. All patients were treated with insulin and none had ketosis, clinical signs of diabetic angiopathy or disorders affecting the cardiovascular system at the time of the examination.

Twenty five patients (12 females and 13 males) ages 17–45 years (mean 31.6 years) with juvenile diabetes mellitus diagnosed before the age of 20 years, of long duration (10–37 years, mean 24.0 years) constituted the long-term diabetic group. All patients were treated with insulin and none had ketosis at the time of the examination. Normal peripheral pulses were present in the lower extremities in all cases. All patients had diabetic retinopathy as evaluated in table 1. Thirteen patients had nephropathy, the diagnosis of which was based on the simultaneous presence of a raised serum creatinine level and proteinuria. Seventeen patients had neuropathy, the diagnosis of which was based on the presence of symptoms and signs of altered sensibility and abolished tendon reflexes in the lower extremities.

A normal maximum blood flow in hyperemic skeletal muscle is the basis for the calculation of the capillary diffusion capacity for sodium as mentioned below (11). In routine clinical evaluations done in this laboratory maximum ^{133}Xe clearance values in the anterior tibial muscle greater than 35.0 ml/100 g/min indicate a normal maximum blood flow in that muscle. Therefore all diabetic patients included in this study were selected to have a maximum ^{133}Xe-clearance value equal to or greater than 35.0 ml/100 g/min.*)

Methods

One tenth ml of normal saline containing 50 µC of ^{133}Xe in solution and 20 µC of ^{24}Na was injected into the thickest part of the tibialis anterior muscle. In one group the two isotope solutions were mixed prior to injection, i.e., the two clearances were measured simultaneously. In a second group the ^{133}Xe clearance was measured first, followed immediately by a measurement of the ^{24}Na clearance. Details regarding the mode of injection have been given elsewhere (11).

The local clearances of ^{133}Xe and ^{24}Na were followed by scintillation detectors coupled to pulse height analyzers and ratemeters with time constants of approximately five seconds. Direct writing logarithmic potentiometers were used. In all clearance studies two 51 mm

*) This series comprises patients from Bispebjerg Hospital, Medical Department II, Statens Blindeinstitut, Hvidore Hospital and Steno Memorial Hospital, Copenhagen. The authors wish to express their gratitude to the chief physicians of the above mentioned institutions for allowing us to examine their patients.

TABLE 1

Maximum clearance rates for ^{133}Xe and ^{24}Na, capillary diffusion capacity for sodium, and time from cuff release to maximum ^{133}Xe clearance in 25 long standing diabetics

Case No	Sex	Age (Yr)	Duration of Diabetes (Yr)	Diabetic Complications			Maximum clearance rates ml/100 g/min		Capillary diffusion capacity for sodium ml/100 g min	Time to max ^{133}Xe clearance in min
				Retinopathy	Nephropathy	Neuropathy	^{133}Xe	^{24}Na		
1	F	45	35	+++	+	+	35.2	14.6	11.1	0.18
2	F	30	20	+++	+	+	79.0	14.0	9.4	0.16
3	F	33	29	+++	+	+	44.0	11.6	10.5	0.30
4	M	25	19	++	+	0	67.0	17.4	12.0	0.20
5	M	45	25	++	+	+	37.2	16.1	12.4	0.13
6	F	31	30	+++	+	+	80.0	13.3	8.7	0.10
7	M	17	10	+	0	0	44.4	14.4	10.3	0.20
8	F	27	12	+	0	0	42.0	15.2	11.1	0.26
9	F	37	28	+++	0	+	43.0	8.8	5.8	0.32
10	M	31	30	+++	+	+	35.9	13.2	9.8	0.08
11	M	25	23	+++	+	+	84.0	16.1	10.6	0.12
12	F	43	31	++	0	+	47.2	16.8	12.3	0.10
13	M	33	22	+++	+	+	42.0	13.1	9.4	0.10
14	F	38	29	+++	0	+	13.0	9.0	6.0	0.28
15	M	26	24	+++	+	+	85.0	11.0	9.2	0.10
16	M	25	19	+	0	0	51.0	12.1	8.8	0.12
17	M	39	36	+	0	+	47.2	10.7	7.2	0.28
18	F	33	16	+	0	0	44.3	11.1	7.7	0.08
19	M	35	17	++	+	+	36.0	11.0	10.5	0.08
20	F	20	17	+	0	0	18.0	11.0	9.9	0.10
21	M	37	29	+++	0	+	52.0	10.5	7.0	0.51
22	M	31	19	+++	0	0	16.0	7.0	4.6	0.60
23	M	20	16	++	+	0	75.0	8.3	5.3	0.50
24	F	42	37	+++	+	+	48.0	12.3	8.5	0.10
25	F	22	12	+	0	+	47.2	12.4	8.6	0.08
Mean		31.6	24.0				52.2	[illegible]	9.1	0.20
SD		8.0					15.9	2.7	2.2	0.15

The degree of retinopathy was evaluated as follows: + … the presence of … ++ … +++ … [illegible]

thick crystals (one for each leg) were used for the ^{24}Na high energy gamma ray detection whereas two 3 mm thick crystals were employed to measure the ^{133}Xe low energy gamma rays

Initial counting rates averaged approximately 5,000 cps for ^{133}Xe and approximately 1,000 cps for ^{24}Na In the simultaneously measured clearance curves a ca 10 per cent correction for the ^{24}Na gamma rays detected by the ^{133}Xe channel was applied In the independent clearance studies no crossover occurred since the sodium channel was completely insensitive to the ^{133}Xe gamma emissions

All patients were studied while lying on their backs with their legs suspended in a fixed position relative to the crystals After the injection of the isotope solution, and after a short rest period, a cuff placed just above the knee was suddenly inflated to a pressure of 250–300 mm Hg

During the induced ischemia the patients moved the ankle joint by doing full force dorsiflexion plantarflexion movements After ca 80–100 movements further exercise was impossible because of pain and muscle fatigue At this point, after 2–5 minutes of ischemia, the cuff s pressure was abruptly released and during the subsequent period of reactive hyperemia the isotope clearance was followed for approximately 5 minutes

Total radiation dosage to the gonads from ^{24}Na is approximately 80 millirads Radiation dosage from ^{133}Xe is negligible (12)

Calculations

The clearance rates

For both tracers the rate of decrease of the concentration per gram of tissue, $dC/dt = C'$, can be expressed by applying the Fick principle to 100 g of muscle

$$100 \; C' = - f \; Cv \qquad (1)$$

where f is the blood flow in millilitres per 100 g per minute in the injected area and Cv the corresponding venous concentration This equation does not contain on its right hand side a term for the rate of supply (f Ca) since recirculation is negligible

The clearance rate, Cl, in milliliters per 100 g per minute, is defined according to Renkin (17, 18, 19) in analogy with the clearance concept in kidney physiology Cl is that imaginary volume of blood in ml/100 g/min that would have contained the amount of tracer leaving 100 g of tissue (f Cv) if complete diffusion equilibrium had occurred At equilibrium $Cv_{equil} = C/\lambda$ where λ is the tissue-blood partion coefficient, i e $\lambda = (C/Cv)_{equil}$ According to these definitions,

$$f \; Cv = Cl \; Cv_{equil} = Cl \; C/\lambda$$

Inserting this in equation (1) one obtains $Cl = 100 \; \lambda \; (- C'/C)$ ml of blood/100 g/min (2) λ_{Na} equals about 0 5 ml/g as it is given by the ratio of the tissue sodium concentration, ca 40 µEq/g to that of whole blood, ca 80 µEq/g (11) λ_{Xe} equals about 0 7 ml/g in blood of normal hemoglobin concentration (3)

$-C'/C$ = the relative rate of decrease of tissue isotope concentration which can be expressed

$-C'/C = -d \ln C/dt = \ln(10)(-d \log C/dt)$, i.e. $-C'/C$ equals the natural logarithm of 10 (2.30) times the slope of the logarithmically recorded curve, this slope being measured in fractions of one decade (see fig. 1).

Time from cuff release to maximum ^{133}*Xe clearance*

As shown in fig. 1 time to maximum ^{133}Xe clearance was read directly in minutes from the time of cuff release to the beginning of the steepest rectilinear part of the ^{133}Xe clearance curve.

Capillary diffusion capacity for sodium

According to Renkin (17, 18, 19) the capillary diffusion capacity for sodium (CDC_{Na}) is defined as the unidirectional flux of the sodium ion from the tissue into the capillary lumen. It is expressed in the unit, milliliters of plasma per 100 g skeletal muscle per minute, i.e. it corresponds to the amount

Sodium-24 and Xenon-133 clearance curves in a healthy subject obtained by means of a logarithmic potentiometer

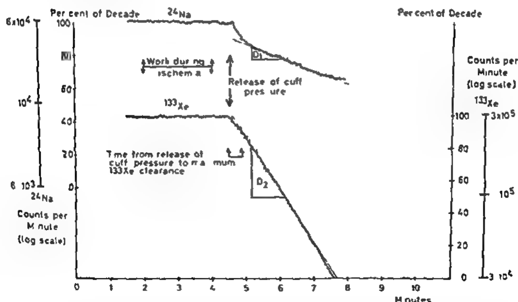


Fig. 1

The graph indicates a period of cuff obstructed blood flow in the leg during which muscular work is carried out for two minutes. The slope D_1 after release of cuff pressure is 8 per cent of one decade on the logarithmic potentiometer. Maximal ^{24}Na clearance is consequently 115 · 0.08 = 9.2 ml per 100 g per min. The maximum slope D_2 is 32 per cent of one decade and consequently maximal ^{133}Xe clearance is 161 · 0.32 = 51.5 ml per 100 g per min. Maximum reactive hyperemia sets in 0.40 min after release of cuff pressure.

of sodium contained in this volume of plasma The CDC_{Na} can be estimated in hyperemic skeletal muscle, where the back diffusion of sodium is small (14) In fact at infinitely high blood flow the capillary concentration of ^{24}Na would remain zero and hence the plasma clearance of sodium would be equal to CDC_{Na}

CDC_{Na} = (1–Ht) Cl_{Na} ml of plasma/ 100 g muscle/min (3)

According to the above considerations only one value of Cl_{Na} was calculated from the recorded curve, viz, the value obtained during the period of maximum reactive hyperemia The time interval during which maximum muscle blood flow prevailed was read from the steepest rectilinear part of the ^{133}Xe clearance curve This period usually lasted from 0 5 to 1 5 min after release of the femoral cuff, and typically the sodium clearance curve was also practically linear during this interval In all cases the curve was therefore approximated by a straight line (see fig 1)

As CDC_{Na} is the unidirectional flux of ^{24}Na , a correction is made for the back diffusion of ^{24}Na from the capillary blood The back diffusion is proportional to the mean capillary concentration of ^{24}Na which under the special conditions studied (viz of fairly incomplete exchange) can be taken to be approximately half of the end capillary concentration of ^{24}Na On this basis it can be shown (13) that if the local blood flow is given by the simultaneous Cl_{Xe} then

$$CDC_{Na} = (1 - Ht)\, Cl_{Na} \; \frac{1}{1 - {}^{1}/_{2}\, Cl_{Na}/Cl_{Xe}}$$

ml/100 g/min

where Ht is the hematocrit of the blood The capillary diffusion capacity as here calculated is expressed in terms of the number of milliliters of plasma whose sodium passes unidirectionally across the capillary membrane in 100 grams of muscle during one minute

RESULTS

All values reported for maximum clearance rates of ^{133}Xe and ^{24}Na, the CDC_{Na} and the time from cuff release to maximum ^{133}Xe clearance are averages of the two values obtained independently from each leg

^{133}Xe clearance

The maximum blood flow in the anterior tibial muscle during reactive hyperemia after ischemia and exercise, as judged by the mean maximum ^{133}Xe clearance, did not differ significantly within the three groups studied here

As shown in table 2 no statistically significant difference in the mean time from cuff release to maximum ^{133}Xe clearance was observed between non-diabetics (0 38 min) and recently-diagnosed diabetics (0 34 min) In contrast a highly significant reduction to 0 20 minutes was observed in this variable in the long standing diabetic group The mean time to maximum ^{133}Xe clearance did not differ significantly be-

TABLE 2

Average maximum clearance rates for ^{133}Xe and ^{24}Na, capillary diffusion capacity for sodium and time to maximum ^{133}Xe clearance in non-diabetics, recently diagnosed diabetics and long-standing diabetics

	Case no.	Age (y)	Duration of Diabetes (y)	Maximum clearance rates (%/min) Xe	Maximum clearance rates (%/min) Na	Capillary diffusion capacity for sodium (ml/100 g/min)	Time to max. Xe clearance (min)
Long-standing diabetics	25	31.6 SD 8.0	24.0	52.2 SD 15.9	12.9 SD 2.7	9.1 SD 2.2	0.70 SD 0.15
Recently diagnosed diabetics	7	19.3 SD 2.8	1.9	60.5 SD 10.4	8.0 SD 1.3	5.2 SD 0.85	0.34 SD 0.12
Non-diabetics	13	29.1 SD 6.5		55.6 SD 9.6	9.9 SD 1.0	6.6 SD 0.80	0.38 SD 0.10
Statistical Analysis							
Long-standing diabetics versus non-diabetics				n.s.	$p < 0.001$	$p < 0.001$	$p < 0.001$
Long-standing diabetics versus Recently diagnosed diabetics				s	$p < 0.001$	$p < 0.001$	$p < 0.001$
Recently diagnosed diabetics versus non-diabetics				n.s.	$p < 0.002$	$p < 0.002$	n.s.

n.s. = not significant

tween various subgroups of the patients with long standing diabetes Parameters according to which these subgroups were selected included age, sex, duration of diabetes and severity of diabetic complications (retinopathy, nephropathy and neuropathy) It is of special interest (see discussion) that no significant difference ($p > 0.5$) was observed in the average time to maximum ^{133}Xe clearance between seventeen long standing diabetic patients with diabetic neuropathy (0 18 (S D 0 17) minutes) and eight patients without this complication (0 26 (S D 0 22) minutes)

²⁴Na clearance

The mean clearance rates for ^{24}Na differed highly significantly between non diabetics (9 9 ml/100 g/min) and recently-diagnosed juvenile diabetics (8 0 ml/100 g/min) on the one hand and juvenile diabetics of long standing (12 9 ml/100 g/min) on the other hand The relatively small, uniform group of patients with recently-diagnosed diabetes had a significantly lower ($p < 0.005$) mean age as well as significantly lower mean ^{24}Na clearance compared to the non diabetic group Subgroups of the patients with long standing diabetes did not differ significantly with respect to mean clearance rates for ^{24}Na

Capillary diffusion capacity for sodium

As shown in table 2 and fig 2 mean CDC_{Na} differed highly significantly between non diabetics (6 6 ml/100 g/min) and recently diagnosed diabetics (5 2 ml/100 g/min) as opposed to juvenile diabetics of long standing (9 1 ml/100 g/min) The mean CDC_{Na} value in the group of patients with recently diagnosed diabetes was found to be significantly lower than in the group of non diabetic patients No significant difference was found between subgroups of the long-standing diabetic patients (see above)

DISCUSSION

An important factor in capillary exchange is the permeability of the capillary membrane, the diffusion capacity of which is known to be strikingly different for different substances Renkin (17, 18, 19) found that potassium and rubidium encounter a distinct resistance to transcapillary diffusion in hyperemic skeletal muscle Nevertheless cations diffuse far more rapidly across the capillary membrane than proteins, the exchange of which is very slow and probably occurs through sparsely distributed large pores (9) On the other hand, the transcapillary filtration rate for water vastly exceeds the diffusion rate for cations

Transcapillary diffusion of sodium in diabetics

Following Renkin (17, 18, 19) the capillary blood flow in hyperemic skeletal muscle is so rapid that the clearance rate of intramuscularly injected ^{24}Na represents a measure of the unidirectional sodium flux from tissue to capillary blood This unidirectional sodium flux is here expressed as CDC_{Na}

and is given in milliliters of plasma per 100 g skeletal muscle per minute, i e the flux of sodium equals the amount of sodium (in mEq) in a given plasma volume

The CDC_{Na} values within the long-standing diabetic group exhibit great variance with some values overlapping into the range of the non diabetic group In this connection it is of interest that the histological studies of skeletal muscle mentioned in the introduction also reported that some patients with clinical signs of severe diabetic complications lacked the characteristic morphological changes of diabetic microangiopathy (2, 16, 26)

We have not been able to find reports of the morphology of skeletal muscle capillaries in recently diagnosed juvenile

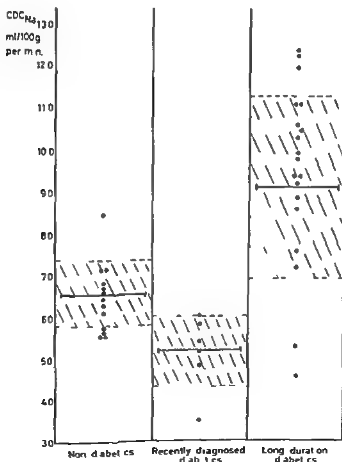


Fig 2

Comparison of capillary diffusion capacity for sodium in non diabetics recently diagnosed diabetics and in diabetics of long duration
observations ● mean value |———| and SD (hatched)

Maximum reactive hyperemia sets in more quickly in the long term diabetics than in the non diabetics or in the recently diagnosed diabetics who not differ significantly It is suggested that this finding is also due to the diabetic micro angiopathy present in the patients with long term diabetes

ACKNOWLEDGEMENT

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COAGULABILITY IN DIABETICS

by

Flemming Valdorf Hansen

The coagulability of the blood is one of the factors which could be considered to play a role in the production of arterial thrombi

Diseases such as coronary occlusion are observed earlier and with greater incidence in diabetics (22) than in non-diabetics, it was therefore considered reasonable to compare the coagulability in these two groups

The first studies appeared around 1950 (9, 10) These suggested that the blood in diabetics possessed an increased power of coagulability, a so-called hypercoagulability Shortened heparin resistance time and increased fibrinogen content in the blood were demonstrated in a group of diabetics whose condition was not specified in further detail

In 1957, Introzzi & de Nicola (13) found an elevated thromboelastographic index in patients with diabetes of old age, and this hypercoagulability in diabetes of mature onset was confirmed by the Modena group (2, 3, 16, 17, 23), among others in 1961 However there were also reports that the coagulability of the blood was normal in such cases (20), and that the coagulability in diabetics was even reduced (11)

One of the best studies from the point of view of coagulation technique was published in 1963 by Egeberg (7) He demonstrated the presence of hypercoagulability in diabetics, and showed that it was due to an increase in the content of the following coagulation factors I (fibrinogen), V (proaccelerin) and VIII (antihaemophilia factor A) Others have found increased factor VII (proconvertin) content in the blood of diabetics (2)

In the majority of studies, no attempts seem to have been made to seek for correlations between diabetes control diabetes duration and diabetic vascular complications Where such studies have been made, the patient groups have consisted mainly of elderly patients with diabetes of a few year s duration, or the information as to the clinical conditions has been inadequate, with limited possibility of revealing possible correlations

AUTHOR S INVESTIGATIONS

Material and methods

Factor I (fibrinogen) was examined by Jacobsson's technique (14) Mean value 223 mg per cent, standard deviation 8

Factor VII (Proconvertin) was determined by a method proposed by Aas (1) Mean value 75 per cent, standard deviation 11

Heparin resistance test · this was performed in special test tubes, clean and unused Daily control tests were made on thawed normal plasma with thawed, standardized heparin The coagulation system consisted of 0 5 ml citrated, platelet rich plasma, + 0 1 ml heparin (10 units/ml) incubated for 6 minutes and 25 mM calcium chloride solution then added The coagulation time was a triple coagulation time determination with pilot test by Waaler's method (24) Mean value 5 11 minutes, standard deviation 0 20

Coagulation studies these were performed blind on coded blood samples at 37 5° C ± 1° All determinations were started within one hour of taking blood samples Blood and plasma samples were stored in an iced water bath and centrifuged in a refrigerated centrifuge Only perfect venepunctures were accepted

Recalcification test · this was carried out and read off essentially as a heparin resistance test The coagulation system consisted of 0 5 ml citrated platelet-rich plasma + 0 5 ml citrate solution (citrate concentration 0 4 meq/liter, ionic strength 0 154, pH 7 35) This was incubated for 6 minutes and 0 5 ml preheated calcium chloride solution was then added Mean value 3 25 minutes, standard deviation 0 17

Silicon treatment needles were siliconed in 1/2 per cent Monocote (Armour lab, Hapden Park, Eastbourne, England) Construction pipettes were siliconed with an 0 2 per cent solution of Dow Corning Z 4141 (Dow Corning *Corporation,* Midland, Michigan, U S A) Silicon treatment was carried out in a special room, pipettes and needles were resiliconed after being used once, and the latter were ground each time before use

Thrombin time determinations these were done as described by Hjort & Stormorkin (12) A daily check was performed with thawed thrombin on thawed normal plasma The coagulation system consisted of 0 2 ml citrated plasma rich in platelets + 0 2 ml citrate solution (see above), incubated for 3 minutes and thrombin solution (3 7 NIH units/ml) then added Mean value 29 seconds, standard deviation 1

Patient material Age distribution and duration of diabetes in the 106 patients is shown in fig 1 and fig 2 As seen, there were few elderly diabetics, only 16 patients (15 per cent) being over 50 years, and of these only 2 over 60 years There were many patients, however, with a long duration of diabetes, 31 per cent of the patients had a duration of diabetes of 25 years or more In 55 patients, the diabetes had started before the age of 16 years Among the other patients, only one was over the age of 40 years when diabetes was diagnosed Only one patient was controlled without insulin

Normal material This was selected so that it corresponded to the diabetic group as far as concerned sex, age and occupation When it appeared that the results of the coagulation studies were independent of the sex and age of the normal subjects, the various groups of dia-

betic patients were compared with the normal material as a whole

Studies in diabetic patients with diabetes mellitus without clinically recognizable complications

This group comprised 17 women and 19 men The mean age was 33 years and the age varied from 15 to 60 years The mean duration of diabetes was 17 years, with a variation from a few months to 43 years Seven patients had had diabetes mellitus for less than 1 year, 7 had had it for 1 to 10 years, [redacted] had had it from 11 to 20 years, [redacted] from 21 to 30 years and 6 more than 30 years The

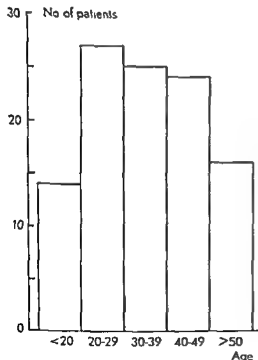


Fig 1

Age distribution of the 106 patients comprising the material

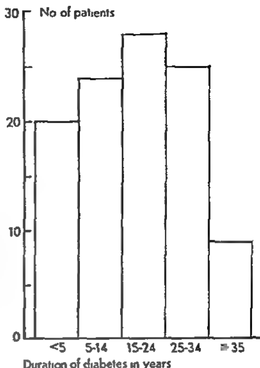


Fig 2

Duration of the diabetes in the 106 patients comprising the material

highest age at which the diabetes had been found was forty years All patients required insulin None suffered from intercurrent disease, and in no case was ketosis demonstrated within the last 14 days prior to the investigation

The mean values for heparin resistance and recalcification time in the diabetes group were respectively, 4 17 minutes and 2 41 minutes The values for normal subjects were, respectively, 5 11 minutes and 3 25 minutes The figures differed to a significant degreee, $p < 0\ 001$ in both cases The mean values for thrombin time, factor VII per cent and fibrinogen content were normal in these patients

The recalcification time was shortened significantly with duration of diabetes, $p = 0.02$. There was also a certain probability that the factor VII content was increased with duration of diabetes. The latter relationship was significant if all values were included, $0.02 < p < 0.05$, but only at the limit of significance, $0.05 < p < 0.10$, if an extreme value was rejected.

No statistically significant relationship was demonstrated between the blood sugar and the results of the coagulation studies.

Studies in patients with simple diabetic retinopathy

Employing the terminology of Ehlers (8), a distinction was made only between proliferative and simple retinopathy.

In the group based on the latter form of retinopathy there were 13 men and 5 women, with a mean age of 40 years and a mean duration of diabetes of 23 years. There were only 5 patients with simple retinopathy as an isolated vascular complication, while 10 of the patients had nephropathy.

The mean values for heparin resistance time and recalcification time were, respectively, 4.51 minutes and 2.81 minutes and were significantly lower than the figures for the normal group, 5.11 minutes and 3.25 minutes, $0.02 < p < 0.05$ and $0.01 < p < 0.02$, respectively. In these diabetic patients, the fibrinogen in the blood was significantly increased in relation to that in normal subjects. The means were, respectively, 312 mg per cent and 223 mg per cent, $p < 0.001$. The results of the other coagulation studies did not differ significantly in the two groups.

The fibrinogen content of the blood was also found to be significantly raised in relation to that in diabetics without clinically recognizable complications. The means were, respectively, 312 mg per cent and 223 mg per cent, $p < 0.001$. The mean of the recalcification times in diabetics with simple retinopathy, 2.81 minutes, was significantly greater, however, than the value in diabetics without clinically recognizable complications, which was 2.41 minutes, $0.002 < p < 0.01$. There were no other significant differences between the results of the coagulation studies in these groups.

Studies in patients with proliferative diabetic retinopathy

This group included 12 men and 8 women. In 16 patients, the visual acuity was $< 6/9$ in both eyes. The mean age was 37 years. The mean duration of diabetes was 20 years. Only 4 patients had proliferative retinopathy as sole complication to the disease. Of the remaining patients, 13 had nephropathy and 6 had uraemia.

The heparin resistance times and the recalcification times were significantly reduced, the means being, respectively, 4.35 minutes and 2.54 minutes for diabetics with proliferative retinopathy, 5.11 minutes and 3.25 minutes for normal subjects, $0.02 < p < 0.05$ and $p < 0.001$, respectively. The fibrinogen content and the factor VII content in the blood were also significantly elevated

Diabetics with proliferative retinopathy had mean values of 362 mg per cent and 98 per cent. The corresponding figures for the normal group were 223 mg per cent and 75 per cent, $p < 0.001$ and $0.01 < p < 0.02$, respectively.

In contrast to the other results, the thrombin times were found prolonged in patients with proliferative diabetic retinopathy. The mean value in these patients was 32 seconds, in normal subjects 29 seconds, $0.01 < p < 0.02$.

In comparison with diabetics without clinically recognizable complications, patients with proliferative retinopathy were also found to have a significantly elevated fibrinogen content, $p < 0{,}001$, the mean value for the latter group being 362 mg per cent, against 223 mg per cent for the former. The mean for the factor VII value in the blood of diabetics with proliferative retinopathy was 99 per cent, which was significantly greater, however, than the mean value for diabetics without clinically recognizable complications, if the extreme value of 350 per cent referred to on page 150 was omitted in the case of the latter group of patients. The other tests showed no significant differences between these groups of diabetics.

Studies in patients with diabetic nephropathy

The criterion used for nephropathy was the definition used by Wilson et al. (25). There were 17 men and 10 women in this group, with a mean age of 35 years and a mean duration of diabetes of 20 years. Only one patient had isolated nephropathy, all the others had vascular complications. Retinopathy was present in 23 patients. Nine of the patients had uraemia.

Just as in the other groups of diabetics, the heparin resistance times and recalcification times were significantly reduced when compared with normal values, the mean values being respectively, 4.26 minutes and 2.58 minutes, against normal values of 5.11 minutes and 3.25 minutes. In both cases, $p < 0.001$. The mean values for the results of the fibrinogen and factor VII determinations were, respectively, 360 mg per cent and 96 per cent, significantly greater than the normal mean values of 223 mg per cent and 75 per cent, p values being respectively $p < 0.001$ and $p = 0{,}01$. The mean value for the thrombin time, 33 seconds, was significantly greater than the normal value, 29 seconds, $p < 0{,}001$.

The fibrinogen content of the blood was also significantly greater in patients with nephropathy than in patients without clinically recognizable complications. The mean values were 360 mg per cent and 223 mg per cent, respectively, $p < 0{,}001$. The factor VII values were only significantly increased in patients with nephropathy if the extreme value mentioned on page 150 was excluded. A comparison of thrombin times in these groups also showed a significant prolongation in diabetics with nephropathy, 33 seconds, in comparison with the value of 30 seconds in diabetics without clinically recognizable complications, $p < 0.001$. The heparin resistance times and the recalcification times did not differ significantly in the two groups.

A relationship was sought between the protein pattern, serum cholesterol

and serum creatinine on the one hand, and the results of the different coagulation studies on the other hand. The sole correlation found showed a relationship between elevated serum creatinine and elevated fibrinogen content in the blood, $0{,}02 < p\ (r = 0) < 0.05$

Studies in patients with diabetic nephropathy without simultaneous proliferative retinopathy

This group of 9 men and 5 women had fewer and less advances vascular complications than the others in the group with nephropathy.

The mean age was 34 years, the mean duration of diabetes 22 years. With some few exceptions, coagulability showed the same features as in the entire group with nephropathy. The mean value of the factor VII content, however, did not differ significantly from that of the normal material.

Comparison with diabetics without clinically recognizable complications also showed no significant difference for the mean value of factor VII per cent, whereas the recalcification times were increased in diabetics with nephropathy without simultaneous proliferative retinopathy. The means for these patients were 2.76 minutes, for diabetics without clinical complications 2.41 minutes, $0.02 < p < 0.05$

Studies in patients with cardiovascular complications

These patients were not primarily grouped according to special criteria. They were mainly patients who apparently did not primarily have diabetic complications, in spite of a long duration of disease. On further examination, however, they were found to have moderate vascular complications, such as changes in the ECG, hypertension or intermittent claudication. To a greater degree than in the groups already studied, the complications in these patients could well be due to changes in the great vessels. For this reason, therefore, they were collected into a special group. The group consisted of 8 men and 7 women. The mean age was 48 years. The mean duration of diabetes was 30 years, 6 of the patients had retinopathy, none had nephropathy.

The only difference between the coagulation in this group and in the group of diabetics without clinically recognizable complications was that these patients had an elevated blood fibrinogen content, 282 mg per cent. This was significantly greater than both that of normal subjects and diabetics without clinically recognizable complications, 223 mg per cent. The p values were $0.002 < p < 0.01$ and $0.01 < p < 0.02$, respectively.

Supplementary investigations

Ten patients were examined prior to and during insulin-induced hypoglycaemia. No significant changes in coagulation were found as a result of any of the methods employed.

Ten patients were also examined during severe ketosis. Seven of them underwent a control examination from 1 to 4 weeks after termination of the ketosis. A test of the urine during the ketosis

showed that the patients had a + Gerhard's reaction According to Poulsen's studies (21), this signifies a ketone content in the blood of at least 11 mg per cent

The factor VII content of the blood was found to be significantly elevated during ketosis, with mean values of respectively 66 per cent and 49 per cent, $0.002 < p < 0.01$ The mean heparin resistance time and recalcification time during ketosis were 4.00 minutes and 2.17 minutes, respectively, and outside the period of ketosis, 4.46 minutes and 2.63 minutes, respectively Calculation of significance showed that the difference between these results lay at the boundary of significance The results of the other coagulation studies showed no significant changes during ketosis

An examination was made of the possible influence of saturated and unsaturated fats on coagulation Nineteen patients were examined before and about 2½ hours after a standard fat rich meal of about 1000 Kcal All patients were subjected to this load of saturated and unsaturated fats, on various days No changes could be found in the coagulation picture as a result of these fat loads

Discussion

The findings in diabetics without clinically recognizable complications are difficult to compare with the results obtained in other investigations Among investigators to date only Angeli et al (4) have examined the conditions in such a group of patients It should be mentioned in this connection that the beds in the Steno Memorial Hospital are occupied mainly by diabetes patients admitted to routine control or to fine adjustment of their diabetes control

In contrast to this, it must be regarded as unusual for diabetic patients to be hospitalized in a medical department without either poor control before admission, or vascular complications of their disease None of the previous studies state that the investigations were made in special hospitals for diabetics In the present material, those patients who on examination (often ambulant) were found to be without clinically recognizable complications, may thus quite well have differed from the diabetic patients examined by other investigators

Angeli et al (4) found normal global tests, elevated fibrinogen content, but prolonged thrombin time, in diabetics without complications These investigators had studied 10 diabetics less than 40 years of age with "severe" diabetes without complications Investigators such as Ottaviani & Redi (20) found completely normal coagulation in diabetics The majority of previous investigators, however, have reported that diabetics had in general reduced global test times Practically all found elevated fibrinogen content in the blood of all diabetics, while only Alberini et al (2) demonstrated elevated factor VII content in 90 per cent of their patients with diabetes of late onset

However, the discrepancies between the different results in earlier coagulation studies in diabetics, and the discrepancies between these results and the results presented here, cannot be explained solely by differences in the clinical condition of the patients, even

though this can contribute to obscuring the matter Technical differences are difficult to evaluate, as in most cases methods were used which are not essentially different

In general, it may be concluded that the shortened global test times found in this study in diabetics without clinically recognizable complications, have not previously been demonstrated with certainty in such patients However, shortened global test times have often been found among groups of diabetics whose characteristics have not been specifically defined On the other hand, increasing shortening of the recalcification time with increasing duration of diabetes has not been demonstrated previously This may have some relation to the fact that in the present study, there were many patients who had a long duration of diabetes, without clinically recognizable complications Egeberg, who had also examined some of these patients, reported that the material included 3 patients with recent, untreated diabetes These may have had a greater or lesser content of ketones in the blood, and may thus have distorted the picture

In the present study, all diabetics with vascular complications had an elevated blood fibrinogen content, while heparin resistance times and recalcification times were of the same order of magnitude as in diabetics without complications In the groups with simple retinopathy and nephropathy without simultaneous proliferative retinopathy the recalcification times were prolonged even by comparison with diabetics without complications In 14 diabetics with one or more unspecified complications localized to the eyes, kidneys, heart of peripheral vessels, Angeli et al found shortened recalcification times This is in poor agreement with the present results, but on the other hand there is good agreement with the present finding that the fibrinogen content of the blood in diabetics with vascular complications is greater than in diabetics without such complications

In the groups classed according to nephropathy and proliferative retinopathy, the coagulation characteristics were found to be very uniform This is natural, as 13 of the patients were included in both groups The increase in factor VII found in these two groups has not been described so far Alberini et al (2), as mentioned, found increased factor VII content in the majority of their patients, but it was stated expressly that none of these had proliferative retinopathy Among other authors who have examined diabetics for factor VII in the blood should be mentioned Egeberg (7) and Ottaviani & Redi (20), the latter also examining 5 patients with proliferative retinopathy Both these last mentioned authors, however, found normal factor VII content in diabetics

The prolonged thrombin time in relation to diabetes without clinically recognizable complications, and demonstrated in the present study, was only found in groups in which all patients had diabetic nephropathy This suggests that the prolonged thrombin time is related to the nephropathy A prolonged thrombin time in patients with vascular diabetic complications has previously been found only by Angeli et al (4) These authors, however, found even

more prolonged thrombin times in diabetics without complications, where the present author found normal thrombin times

One study has been made on the coagulation characteristics of patients with diabetic ketosis In their 4 patients, 2 of whom also had cerebral apoplexy, however, Cantelli et al (6) found an increased blood coagulation power when measured by thrombo-elastography An imal experiments (5, 19) have also shown that prolonged ingestion of ketone substances results in increased coagulability of the blood On the other hand, medicament induced acidosis of brief duration has not been found to result in changes in the coagulation characteristics (15) The patients in the present study had had their ketosis for varying lengths of time, presumably often for weeks In spite of this, there were only modest changes in the coagulation characteristics

It is quite uncertain whether the demonstrated changes in the coagulation characteristics have any significance for the greater incidence of arterial thrombi in diabetic It should be mentioned, however, that more recent studies (18) suggest that fibrinogen has an influence on the platelet adhesiveness This is presumably one of the decisive factors for thrombus formation in the circulating blood

SUMMARY

A study is presented of coagulation in 106 diabetics and a comparable normal material The majority of the patients were juvenile diabetics, and 1/3 had a duration of diabetes of 25 years or more Reduced recalcification times and heparin resistance times were found in all diabetic groups

The recalcification time was shortened with increasing duration of diabetes, but the results of the global tests were independent of the vascular complications of diabetes The factor VII content was normal in the group of diabetics without clinically recognizable complications However, if all values including an extreme observation were used in the calculation, the factor VII content in the blood was increased with duration of diabetes The factor VII content was increased both in diabetics with nepropathy and in patients with diabetic ketosis

The fibrinogen content was normal in patients with uncomplicated diabetes mellitus, but increased in all patients with one or other form of vascular diabetic complications

The thrombin times were prolonged in patients with diabetic nephropathy These patients, however, when evaluated by all other tests, were in a hypercoagulable state

TABLE I

Review of results of coagulation studies in diabetics

	Normal group	Diabetics without recognizable complications	Diabetics with simple retinopathy	Diabetics with proliferative retinopathy	Diabetics with nephropathy	Diabetics with nephropathy without proliferative retinopathy	Diabetics with cardiovascular complications
Mean age	35	33	40	37	35	34	48
Duration of diabetes		17	23	20	20	22	30
Recalcification time (mins)	3 25	2 41[1])	2 81[1 3])	2 54[1])	2 58[1])	2 76[1])	2 47[1])
Heparin resistance time (mins)	5 11	4 17[1])	4 51[1])	4 53[1])	4 26[1])	4 40[1])	4 46[1])
Thrombin time (secs)	29	30	30	32[1])	33[1 2])	32[1 2])	29
Factor VII (%)	75	73	79	99[1 2])	96[1 3])	93	75
Fibrinogen content (mg%)	223	223	312[1 2])	362[1 2])	360[1 2])	329[1])	282[1 2])

[1]) Results significantly different from the normal group
) Results significantly different from the group of diabetics without clinically recognizable complications
) Dependent on extreme observation

NB The recalcification times and the factor VII % are compared with diabetics without clinically recognizable complications with a mean duration of disease of 26 years

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LONG-TERM EXPERIMENTAL INSULIN-DEFICIENCY DIABETES

— A MODEL OF DIABETIC ANGIOPATHY?

by

Knud Lundbæk, T. Steen Olsen, H. Ørskov
and *Ruth Østerby Hansen*

In those parts of the world where insulin treatment and well organized medical care are available, the fate of the diabetic patient depends on the rapidity with which diabetic angiopathy develops, and on its severity. There is some clinical evidence that meticulous attention to the control of the metabolic abnormality affords a certain degree of protection against angiopathy, but the great majority of apparently reasonably well controlled patients nevertheless end up with severe eye lesions, and ultimately perish from vascular disease of the kidneys, heart and central nervous system.

The role of hypoinsulinism—relative or absolute—in the development of diabetic angiopathy is still unknown. The study of patients with hypoinsulinism not due to diabetes mellitus, e.g. pancreatectomized patients, is difficult and has led to contradictory results. It follows, therefore, that several attempts have been made to produce diabetic angiopathy experimentally by inducing a state of hypoinsulinism in animals. The results of these studies have shown that lack of insulin leads to the development of severe changes in the kidneys, an *insulin deficiency nephropathy*. Much less is known about the state of the retina and the nervous system in experimental diabetes, mainly because relatively few extended studies have been published in these fields.

In the following a survey will be given of our own findings in experimental diabetes, together with a review of the relevant work published from other laboratories.

THE FINDINGS

The kidneys

Most studies of experimental nephropathy have been performed in *rats*. In 1950 Beveridge & Johnson (6) published a study of a large group of alloxan diabetic rats after 7 to 22 months of diabetes. The interpretation of their results is difficult. In all of their diabetic rats on a basal diet they noticed "pyelonephritis" (chronic inflammatory cells, in-

creased amount of fibrous tissue, dilated tubules), however, similar changes although less marked, were found in no less than two thirds of their control animals More or less completely hyalinized glomeruli were seen frequently, but the authors do not specify the incidence of this abnormality in diabetic and non-diabetic animals Foglia et al (14) induced diabetes in rats by pancreatectomy, and found glomerular lesions in 88 per cent of the animals after 2–12 months of hyperglycemia The mesangial areas were increased and the walls of the capillaries were thickened There were no noticeable alterations of the afferent arterioles, other vessels of the kidney or of the interstitial tissue Mann et al (24) found clear-cut abnormalities in the glomeruli of untreated alloxan diabetic rats after 6 to 30 months of diabetes They emphasized that the changes observed differed from those of human diabetic nephropathy, by the absence of collagenous tissue formation In 1959 Janes (18) reported on the occurrence of diffuse and localized thickening of the basement membrane of the glomeruli in alloxan diabetic rats after 3 months, and severe changes after 6 months Greenberg (17) reported "capillary basement membrane thickening" after 3 months of alloxan diabetes Later on, the changes were more severe and the occurrence of localized, pale staining masses was noted, as well as fusion of capillaries Rather late in the disease the lesions became PAS positive The walls of the arterioles were thickened after 8 months, but were without PAS positive material

From our laboratory, Ørskov et al (28) described the structure of the glomeruli in alloxan diabetic rats The experiments were carried out on rats intravenously alloxanized with and without temporary renal clamping The study included several control groups, e g with and without different kinds of insulin, with and without renal clamping, etc Macroscopically the kidneys of the diabetic animals were enlarged The average weight of the kidneys of the diabetic rats was greater than that of the non diabetics Lesions occurred after 10 months of experimental diabetes and were more severe after 15 months (Table 1) The early change consisted of diffuse, fairly uniform PAS-positive thickening of the glomerular capillary walls and widening of the mesangial re-

TABLE 1

Glomerular changes in relation to duration of diabetes

Grades of severity	Controls	Diabetic rats Duration of diabetes			Total
		5 mo	10 mo	16 mo	
+++	0	[illegible]	0	5	5
++	0	0	3	[illegible]	5
+	1	0	12	0	13
0	35	22	5	0	62
total	36	22	20	7	

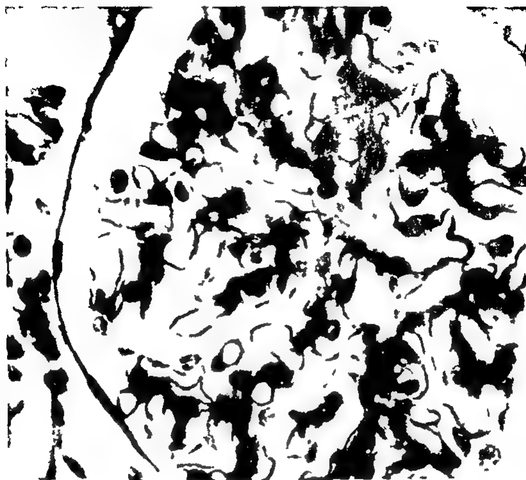


Fig 1

Glomerulus from a rat with diabetes of 10 months duration The glomerular lesion is diffuse of moderate degree

gions (Fig 1) This lesion was very similar to the diffuse glomerulosclerosis of human diabetes The more advanced stages showed essentially the same process but in the severest cases a localized, PAS positive, hyaline substance was also seen (Fig 2) The accumulations were 'sausage shaped , and the impression by light microscopy was that the capillary lumina were filled with cellular and acellular masses Typical nodules of the kind present in human diabetes were not observed

tered (Østerby Hansen et al , 1967)

The results obtained did not suggest that administration of insulin or the alloxan injections were responsible for the glomerular lesions The changes observed in the diabetic animals were not present in age matched controls

However, later experience has shown that somewhat similar glomerular ab-

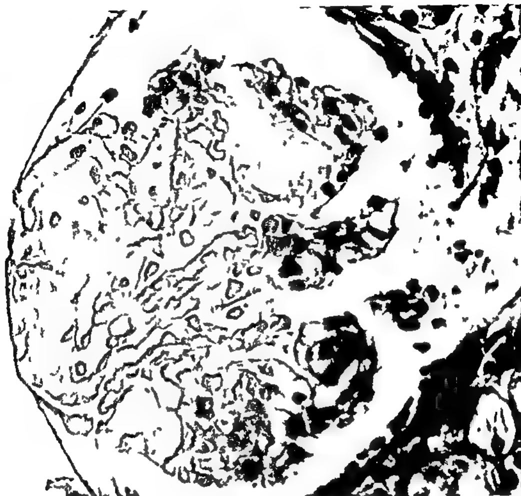


Fig 2

Glomerulus from a rat with alloxan diabetes of 15 months duration The maximal degree of glomerular lesion encountered

normalities occur in *very old* non diabetic rats 24 months), although the severest degrees have not been encoun-

Histochemical investigations of this glomerulopathy in diabetic rats (Steen Olsen et al (27 a) showed that the deposits consisted of a glycoprotein which did not contain glycogen, amyloid or acid mucopolysaccharides Lipid was demonstrated in a few glomeruli with pronounced changes The severest lesion with "sausage shaped' deposits showed histochemical similarity to the so called exudative lesions (capsular drop, fibrinoid cap and arteriolar hyaline) of human diabetic glomerulopathy Experimental lesions of milder degree showed both structural and histochemical similarity to the human diffuse lesion

A number of the diabetic rats in our study showed moderate interstitial fibrosis and lymphocytic infiltration as well

as dilated tubules with protein casts and epithelial degeneration These changes were most pronounced in a group of rats in which the kidneys were not protected against the alloxan by clamping off the renal vessels Thus direct alloxan damage seemed to be an important factor in the development of tubular and interstitial changes However, it is likely that part of these changes were secondary to the glomerular lesions (ischemia in areas supplied with blood from the degenerating glomeruli)

A few reports have appeared concerning kidney changes in other animals with experimental diabetes

Lukens & Dohan (23) described a case of experimental pituitary diabetes lasting for 5 years in a *dog* At autopsy, typical Kimmelstiel Wilson lesions were seen in the glomeruli Ricketts and coworkers (35) studied the kidneys in 10 dogs made diabetic by subtotal pancreatectomy, and 8 treated with pituitary extract after subtotal pancreatectomy After 1–8 years' observation, diffuse glomerulosclerosis was noted in well controlled as well as in poorly controlled animals The glomerular lesion was described as a thickening of the intercapillary stroma and of the capillary walls Similar changes occurred in the controls which were older than the diabetic animals, but much less frequently Lipid deposits in the glomeruli were noted in poorly controlled diabetic dogs True nodular changes were never seen

Beaser et al (2, 3) followed the development of the kidney lesions in alloxan diabetic golden *hamsters* for up to two years They observed glomerular changes similar to the diffuse form of human glomerulosclerosis, increasing in severity with the duration of diabetes In addition they noted the appearance of a "pyelonephritis" quite similar to the changes we observed in the interstitual tissue of our alloxan diabetic rats They do not present evidence that these changes were caused by a chronic bacterial infection of the renal parenchyma They emphasized the absence of significant changes in the vascular walls of the small renal vessels, a finding compatible with ours The nephropathy in these hamsters was more severe in animals poorly controlled with insulin In older control animals they found "stiffening of glomerular walls , a picture differing from that of the diabetic animals

Bloodworth (8) gave a detailed report of his investigation on 10 dogs made diabetic with pituitary growth hormone or alloxan, and maintained with insulin for 1–5 years Diffuse glomerulosclerosis developed in all the dogs, as described by Ricketts et al (35), but in contradistinction to the findings of these investigators, Bloodworth found nodular changes, identical to those of severe human glomerulosclerosis, in 7 of the 10 diabetic dogs but in only one of 9 control animals He also noted other glomerular changes characteristic of the spontaneous disease in human beings, such as exudative lesions, arterial sclerosis, and tubular changes

Summarizing the light microscopic observations of several authors, it can be stated that it is possible to induce glomerular changes in various laboratory animals by means of insulin deficiency diabetes In most cases this lesion is diffuse and consists of a PAS positive thickening

of the capillary wall as well as an increase of the mesangial areas This stage is similar to diffuse diabetic glomerulopathy in the human There are some differences of opinion as to the time required to produce these lesions, a fact which may be explained by differences in experimental conditions and species In the rat, the most severe diabetic lesions appear as focal, partially fusing, homogenous PAS-positive areas suggesting deposits in capillary loops These areas do not have the same appearance as nodular Kimmelstiel-Wilson lesions in the human subject Histochemically they are similar to the so called exudative lesions in human diabetic nephropathy In the dog it seems to be possible to produce nodules very similar to those of the human disease In striking contrast to what happens in the diabetic patients, most experimental animals do not develop severe PAS-positive thickening of the juxta glomerular arterioles Also here the dog constitutes an exception

There are only few reports in the literature on *electron microscopic* studies of experimental glomerulopathy

Bloodworth in the paper discussed above (8) described the ultrastructure of the glomeruli in dogs with experimental diabetes The mildest change was a thickening of the basement membrane He did not measure the basement membrane but estimated it to be 2–4 times thicker than in normal animals The more advanced stage was characterized by an abnormal amount of basement membrane material in the mesangial areas The author thinks that this process, which leads to the development of typical Kimmelstiel-Wilson nodules, is essentially the same in the dog as in diabetic patients

Gibbs et al (16) have published a short report on their electron microscopic findings in alloxan diabetic *monkeys* In one animal, after 73 months of diabetes, accumulation of basement membrane-like material in the mesangial areas of the glomeruli and thickening of the peripheral basement membrane was observed Similar, although rather less marked alterations were found in some animals with diabetes of 19–55 months' duration High carbohydrate diet or low insulin dosage seemed to favour the development of these changes

In our electron microscopic studies of glomeruli from alloxan diabetic rats (Østerby Hansen et al (29)) the findings by light microscopy were confirmed, i e thickening of the capillary walls and increase in mesangial areas Furthermore, several ultrastructural abnormalities were encountered We have observed localized 'spikes' on the epithelial side of the peripheral basement menbrane, occurring rather constantly in pairs with a more electron dense material in the basement membrane between two adjacent spikes (Fig 3) Reconstructions based on serial sections showed that these profiles represented 'moon crater' like formations on the epithelial surface of the basement membrane A more diffuse thickening was present in some glomeruli In some cases a definite increase in mesangial areas was observed, sometimes consisting of cellular elements (Fig 4), sometimes made up mainly of basement membrane material This material showed increased electron density

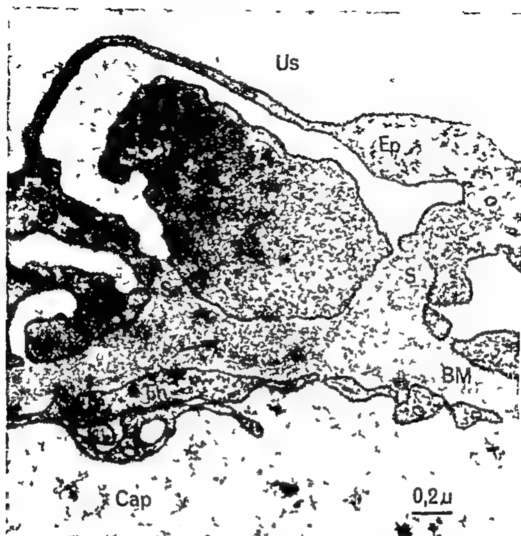


Fig 3

Section through a moon crater formation on the glomerular basement membrane (Bm) Two spikes (S) are projecting towards the epithelial cell (Ep) An electron dense fibrillar material is seen in the basement membrane between the spikes (arrow) and in the cytoplasm of the epithelial cell immediately adjacent (En) endothelial cell (Us) urinary space (Cap) capillary lumen Vestopal embedding Uranyl acetate stain

in some of the diabetic animals The epithelial cells were severely altered, showing accumulations of large inclusions giant lysosomes, and in the severest cases containing closely packed Golgi complexes and cisterns of endoplasmic reticulum Fusion of foot processes was present in only a few glomeruli

The "moon craters" on the epithelial side of the basement membrane have not been described before in human or experimental diabetes, or in any other



Fig 4

The electron microscopic picture of a part of a sausage shaped accumulation (compare Fig 3) Several mesangial cells (M) and basement membrane branches (Bmb) constitute a large area in a capillary loop close to the capsule of Bowman (Bow) Vestopal embedding Double staining with uranyl magnesium acetate and lead citrate

condition but similar changes have been observed recently in biopsy specimens from diabetic patients (Østerby Hansen (31)) The mesangial increase in basement membrane material as well as the diffuse thickening of the peripheral basement membrane found by Bloodworth in dogs, Gibbs et al in monkeys, and Østerby Hansen et al in rats, seem to be very similar to the electron microscopic lesion in human diabetic glomerulopathy

The eyes

Alloxan diabetic rats and rabbits regu

larly develop severe cataracts in the course of a few months (Bailey et al (1)—Bellows & Shoch (4)—Patterson (32)—Naidorff et al (27)—Bounds et al (9))

Berggren & Brolin (5) tried to provoke abnormalities in retinal vasculature in alloxan diabetic rabbits by exposure to low oxygen pressure Increased capillary tortuosity appeared after the procedure in diabetic as well as in non-diabetic animals No microaneurysms were observed The duration of diabetes in these animals was only 3–12 weeks

Gerritzen et al (15) found no changes in the eyes of 5 alloxanized rats after 11 months of diabetes

Janes & Bounds (19) have published a detailed description of the anatomy of the blood vessels of normal rat eyes In 1957 Janes & Ellis (20) gave a report of their studies of the ocular changes in alloxan diabetic rats They noticed the occurrence of engorgement and dilatation of the iris blood vessels, eventually leading to hyphema and proliferation of corneal vessels These changes were always preceded by cataract formation In a later paper (Janes (18)) the author also described the condition of the retina in alloxan diabetic rats On ophthalmoscopy, retinal hemorrhages were observed in only one of 200 animals However, the time of observation was only 2–3 months Retinitis proliferans-like changes were seen in injected specimens from 2 rats A more common finding was a failure of the retinal capillaries to fill normally during injection It was found that the ocular changes—especially in the anterior segment—were closely related to glomerular abnormalities in the kidneys, as described above

Kirchner & Leopold (21) also applied an injection technique, studying flat preparations of retinae from alloxan diabetic rats injected with silver nitrate Their brief report concerns a pilot study of 12 rats with a duration of diabetes of 3 months Six of the rats were given a high fat diet and in 4 of these the retinal preparations showed a "localized reticular thinning" of the capillary walls

Musacchio et al (25)) studied the vascular tree of the retina in rats after 6–16 months of alloxan diabetes They found a pronounced venous and arterial dilatation and mention "papillary vessels or vascular stars", without describing these phenomena in detail In a later paper (26) they confirmed the dilatation and reported the finding of arterial aneurysms in 2 out of 3 severely diabetic rats The changes observed were correlated to the degree and duration of diabetes

Levene et al (22) reported a study of the retina in 10 "95 per cent pancreatectomized rats The diabetic state of the animals was characterized by an abnormal glucose-cortisone-tolerance test The animals survived without insulin treatment for as long as 27 months Only minor abnormalities developed in these old rats The positive findings were thickening and hyalinization of arterioles seen in ordinary histological sections and increase in PAS-staining of arterioles in trypsin digested, flat preparations of retinae In control rats less than 20 months old, no abnormalities were observed



Fig 6 a

Normal retinal capillary system Indian ink injection specimen Retinal flat preparation

diabetic rats The cataract is probably identical with the human diabetic cataract but there is no precise correlate in human pathology to the anterior segment abnormalities in rats They may be caused by changes in the vessels at the root of the iris perhaps similar in kind to those of the iris vessels producing rubeosis iridis in human diabetic patients

The typical diabetic retinopathy seen in long term diabetic patients does not develop in alloxan diabetic animals Severe phlebopathy haemorrhages and soft or hard exudates are unusual findings A few microaneurysms have been observed by some investigators The very peculiar capillary tangles mentioned above sometimes combined with retinal degeneration have not been described before

It is noteworthy that the entire series of ocular abnormalities observed in long term diabetic rats in our laboratory have also been observed in very old non diabetic rats in the same way as the kidney lesions described above

Peripheral nerves

Bischoff (7) has studied the ultrastructure of the Schwann cells in hamsters 4 weeks after alloxanization He observed severe cytoplasmatic degeneration with lipoid inclusion bodies Similar studies in acute human diabetes are not available

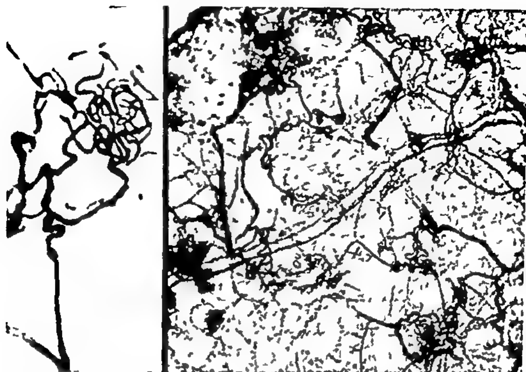


Fig 6 b

Numerous capillary tangles from a rat after 10 months of diabetes Indian ink injection Retinal flat preparation Left part capillary tangle at higher magnification

The brain

In a small series of long term diabetic rats, Reske-Nielsen et al (31) observed degeneration of the brain, as well as many open capillaries with strong PAS positive walls and focal thickenings These changes also occurred in age matched control animals, but were more pronounced in the diabetic rats Pseudocalcinosis of the corpus striatum was present in 2 out of 7 diabetic rats, but in none of 5 controls

The brain abnormalities noted have some similarity to those described in long term juvenile diabetics (Reske Nielsen et al (33)), but the study has to be extended to larger groups of animals

THE SIGNIFICANCE OF THE FINDINGS

There is no doubt that alloxan diabetes and other types of experimental insulin deficiency diabetes lead to the development of a severe and widespread disease in rats and some other animals The evidence available on the kidney changes is particularly convincing undoubtedly because more attention has been paid to this organ than to any other Controlled studies have shown that these changes are not due to the toxic action of alloxan (28)

The question is whether these vascular abnormalities are to be regarded as the animal correlate of diabetic angiopathy

in human subjects Obviously there are considerable differences, especially in the ocular changes, and even the very similar changes in the kidneys are not quite identical to those in human glomerulosclerosis

The point at issue is, of course, to what extent it is permissible to argue about the pathogenesis of diabetic angiopathy on the basis of these experimental findings

If the classical diabetic glomerulosclerosis and retinopathy had been produced in experimental insulin deficiency diabetes in animals, this would certainly have been a very strong argument in favor of the hypothesis that vascular changes developing in diabetic patients are *caused by* lack of insulin On the other hand, it would have afforded an argument *against* the hypothesis that the proneness to develop angiopathy is a primary part of the inherited trait of diabetes mellitus

As a matter of fact the experimental diabetic angiopathy has some similarities to, but is definitely not identical with, human diabetic angiopathy However, even if the pathogenesis of experimental insulin deficiency angiopathy and clinical diabetic angiopathy were the same, absolute morphological identity could hardly be expected

At any rate, in our opinion, the existence of a severe angiopathy in long-term alloxan diabetic animals must be accepted as a positive argument thrown into the scale in favour of the hyphothesis of secondary angiopathy The importance of this interpretation—practically as theoretically—is too obvious to need further elaboration

Our finding that changes similar to those produced by long term insulin deficiency in alloxan diabetic rats are also present in *very old* non diabetic animals is very puzzling It seems to lead us directly back to the old clinical concept of the twenties and thirties, that vascular disease in diabetic patients is "merely an enhancement of the aging processes"

This concept is, of course, untenable to day in view of the fact that old non-diabetic patients actually do not develop diabetic retinopathy, nephropathy, etc On the other hand, the important results of modern survey studies of whole populations (10, 11, 36) may have some significance for the present situation in the field of experimental angiopathy It is now well known that diabetes mellitus, in the sense of a defined carbohydrate intolerance, increases in incidence as people get older Our very old control rats were not studied for evidence of decreased carbohydrate tolerance They did not have glycosuria, but it is a well established fact that abnormal glucose tolerance is very common in old people without glycosuria

It is hardly possible to make meaningful the question whether a female white rat kept in a single cage in a laboratory for 2 years is "older" than an old man or women in our present society Nevertheless, in a speculative mood, one might consider the possibility that the changes observed in the two-year-old non diabetic rat are the equivalent of the retinopathy etc, which *would* develop in old people (the majority of them) who have a diabetic glucose tolerance curve, *if* they lived on for another 20 or 40 years

But quite apart from the argumenta-

tive force of the findings discussed in the field of pathogenesis, and apart from speculations, it seems to us that further comparative work on long term insulin deficiency diabetes and on animal senescence offers reasonable chances of producing interesting new results and concepts in diabetology

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CAUSES OF PERINATAL DEATH IN DIABETIC PREGNANCY

A CLINICO PATHOLOGICAL ANALYSIS

by

Lars Molsted Pedersen and *Jorgen Pedersen*

Analysis of perinatal deaths is hampered by the fact that the cause of death in stillborn babies can only rarely be demonstrated Some have severe congenital malformations, but most of them do not show any anatomical lesions at autopsy Besides, many such infants are macerated or are not autopsied for other reasons In contrast, consecutive autopsy studies of infants dying during the neonatal period are valuable

To obtain as much information as possible about perinatal deaths, a clinico-pathological analysis is necessary in which information about clinical events and conditions of pregnancy and delivery is combined with that obtainable at autopsy

While several causes contributing to the high perinatal mortality in diabetic pregnancy are known (3, 5, 6, 8), it is still a common opinion that unsuspected and unexplainable foetal and neontal death is seen more frequently in offspring of diabetic mothers than in a non diabetic population In order to investigate this, autopsy records and records of complications during pregnancy, delivery and the neonatal period have been analysed in a consecutive series of perinatal deaths in infants of diabetic mothers The complications and causes of death in two decades have been compared, finally, an analysis has been made of causes of death as demonstrated at autopsy in a recent consecutive series of infants dying neonatally

MATERIAL AND METHODS

During the 20-year period 1946–65, 167 of the 846 infants born of diabetic mothers (birthweight 1000 g and more) in the Royal Maternity Department B, Rigshospitalet, Copenhagen, died in the perinatal period 136 diabetic women had one, 11 had two, while 3 women each had 3 infants dying perinatally The period of supervision and treatment during pregnancy varied greatly

The case records of mother and infant as well as the autopsy record of the infant were carefully studied with respect to complications which might explain or help to explain perinatal death Furthermore, one of us (J P) has been con

sultant in diabetes to the department during the 20-year period and personal notes had been taken of several of the infants dying perinatally

The total material of 167 infants comprised 98 stillborn and 69 neonatal deaths However, since microscopy of the organs in infants dying neonatally was first started as a routine in 1960, the consecutive neonatal autopsy material comprises only 35 infants from the period January 1960 to October 1966

DEFINITIONS

Perinatal mortality Stillbirths and neonatal deaths within 10 days after delivery of babies weighing 1000 g and over

Fatal congenital malformations Those which sufficiently explain intrauterine death or are incompatible with extrauterine life for more than 10 days The occurrence of congenital malformation in offspring of diabetic mothers has been discussed in a previous paper (9)

Premature Birthweight less than 2500 g

Pre-coma Diabetic acidosis with a venous plasma standard bicarbonate below 10 meq/l

Severe acidosis Venous plasma standard bicarbonate 10–17 meq/l

Toxaemia 2 of the following 3 signs present

1) B P $\geq$ 140/90 for at least 3 days before delivery
2) More than 0 05 per cent albuminuria for at least 24 hours before delivery
3) Moderate oedema or weight gain $\geq$ 15 kg

Clinical pyelonephritis Urinary tract infection with an acute elevation of temperature exceeding 39° C, confirmed by culture of the urine

Neglectors This term covers three groups of diabetic women Those a) in labour at first attendance in the department, b) with psychopathy or mental retardation, c) with poor social conditions and attending late in pregnancy

PBSP (Prognostically Bad Signs during Pregnancy) *classifications* (8) This classification comprises one or more of the following—just mentioned—signs occurring during pregnancy 1) Clinical pyelonephritis, 2) precoma or severe acidosis, 3) toxaemia and 4) "neglectors" In contrast to White s (10) widely used classification of pregnant diabetics, which is based on factors present prior to the occurrence of pregnancy, the PBSP classification includes complications which become evident during pregnancy

RESULTS

Table I shows the results of the clinico-pathological analysis In 98 infants or nearly 60 per cent, one or more complications were found which fully explain death In 54 infants, or about 30 per cent, complications contributing to death were found, whereas in 15 infants or about 10 per cent, no complications or cause of death could be demonstrated In the last mentioned group, death was as unexplained as it was unexpected

Comments to Table I Each infant is entered under only one heading, even though 2 or more of the complications in question occurred in 45 per cent

Fatal obstetrical complications include 4 groups A) 7 large infants

TABLE I

Clinico pathological analysis of 167 perinatal deaths in infants of diabetic mothers

Complications	Causes of death					
	Primary		Contributing		None	
	No	Per cent	No	Per cent	No	Per cent
Fatal congenital malformation	23	13 8	–	–	–	–
Obstetrical complication	24	14 4	–	–	–	–
PBSP	47	28 1	31	18 5	–	–
Bad obstetrical history	–	–	9	5 5	–	–
Other	4	2 4	14	8 3	–	–
None	–	–	–	–	15	9 0
Total	98	58 7	54	32 3	15	9 0

(birthweight more than 4000 g) in whom difficulties with delivery of the head and /or severe shoulder dystocia occurred Forceps were used in 5 cases, while compression of the umbilical cord occurred in 2 deliveries B) In 6 vaginal deliveries a variety of obstetrical operations were used on account of abnormal presentations or positions C) Abruptio placentae (3), prolapse of the cord (2) and placenta praevia (1) account for six additional deaths, while D) the last 5 fatal obstetrical complications comprise induction of labour extended over several days and/or complicated labour followed by intrauterine or early neonatal death

With PBSP complications as a primary cause of death, infants died in direct connection with the occurrence or aggravation of one or more of the complications, either in utero, or neonatally after onset of spontaneous labour

As a primary cause of death other complications accounting for four deaths were kernicterus (1), sepsis (1), thrombocytopenia hereditaria (1) and severe hepatitis in the mother (1)

In the next column, PBSP complications did not occur directly connected temporally with death, nevertheless the pregnancy was 'marked , since perinatal mortality ■ more than 30 per cent in pregnancies in which PBSP occur, as opposed to 7 per cent in those without PBSP being present (8)

The group bad obstetrical history includes 9 women who in their diabetic state had borne only infants dying perinatally, a group of diabetic women known to have a greater incidence of foetal death, although the cause is unknown (5)

"Other complications in this column comprise 5 pregnancies in which identical circumstances appeared Uncertainty of the stage of pregnancy and entirely wrong estimation of the size of the foetus—hence labour was induced either too late or too early with subsequent intrauterine death or delivery of a very premature baby, respectively 3 pregnancies belonged to White s class F without additional complications, while a variety of complications accounted for a further 6 pregnancies

In 15 women (9 per cent) without major complications, the course of preg-

nancy and delivery was uneventful, but perinatal death occurred quite unexpectedly 5 infants with a birthweight of 2500 g or more died neonatally with symptoms of respiratory distress 10 infants died in utero from 7 to 3 weeks before calculated term Only two had a birthweight less than 2500 g, and no common features could be found for the 10 infants

In Tables II and III the material has been divided into 2 ten year periods, 1946 to 55 with a total of 285 infants including 63 perinatal deaths, and 1956 to 65 with a total of 561 infants including 104 perinatal deaths The incidence of fatal congenital malformations and obstetrical complications, respectively, is compared in the two periods (Table II) Over 10 years, congenital malformations have doubled, while obstetrical complications as a cause of death have decreased to one fifth

The incidence of PBSP was almost identical in the two periods (Table III), but there are marked differences between the four groups constituting PBSP Clinical pyelonephritis and toxaemia have increased while precoma and the group "neglectors" have decreased through the years

In table IV we have compared our material of autopsied infants dying neonatally during the period 1960–65 with a corresponding six-year material (1959–64) from Boston Lying in Hospital (7) Several interesting features can be observed in this table There is no significant difference in neonatal mortality, in the Boston material, however, the neontal period was 30 days The distribution of causes of deaths is similar in the two ma-

TABLE II

Fatal congenital malformations and obstetrical complications in two periods (material 167 infants)

Period	No of deaths	Fatal congenital malformations		Fatal obstetrical complications	
		Per cent of			
		Deaths	Total no of infants	Deaths	Total no of infants
1946–1955	63	7 9	1 7	27 0	6 3
1956–1965	104	17 3	3 2	6 7	1 2

TABLE III

Prognostically bad signs during pregnancy (PBSP) in two periods (material 167 infants)

Period	No of deaths	Clinical pyelonephritis	Toxaemia	Precoma or severe acidosis	Neglectors	Total
		Per cent of death				
1946–1955	63	3 2	11 1	25 4	9 5	49 2
1956–1965	104	8 7	19 2	13 5	3 1	44 5

terials, with infections as one possible exception

The combined material undoubtedly gives a true picture of the causes of neonatal death during recent years Half the deaths were due to hyaline membrane disease and other respiratory problems, one fourth of the deaths were due to congenital malformations and one fourth to other causes including infection, extreme prematurity and birth injury

DISCUSSION

The classification of perinatal death (Table I) is mainly based on the clinical circumstances that precede death Information from the autopsy records was only used in the case of kernicterus and in a few cases of congenital malformation Death was unexplained (and unexpected) in 9 per cent of the infants dying, or in 1 8 per cent of the total number of infants of diabetic mothers Baird et al (1) studied 1008 infants dying perinatally in a maternity hospital during the period 1938 to 1952, and Fairweather et al (4) studied 549 infants from an English city in the period 1960 to 1962 Both studies used a clinico-pathological classification which in many respects was similar to that applied in the present study In the two materials, unexplained death in relation to the perinatal mortality was 33 and 18 per cent, respectively, as compared to 9 per cent in our material, and 1 3 and 0 7 per cent, respectively, as a percentage of the total number of infants, as compared to 1 [illegible] per cent in the diabetic material (Table V)

In contrast to the general opinion, it would appear that the incidence of unsuspected and unexplainable perinatal deaths is no higher in diabetic pregnancy than in the population at large The erroneous general opinion has no doubt been influenced too much by the high perinatal mortality in infants of diabetic mothers

The decrease in fatal obstetrical complications, precoma and acidosis during the last decade illustrates the better management and control of pregnant dia

TABLE IV

Primary cause of neontal death at autopsy²)

Cause of death	Boston material¹) 39 deaths (8 per cent) among 473 infants	Copenhagen material 35 deaths (9 per cent) among 385 infants	Materials combined
Hyaline membrane disease	18 (46)	15 (43)	33 (45)
Respiratory problems (pneumonia pulmonary haemorrhage atelectasis)	3 (8)	5 (14)	8 (11)
Congenital malformation	10 (26)	11 (31)	21 (28)
Infection	[illegible] (15)	1 (3)	7 (9)
Extreme prematurity	1 (2 5)	1 (3)	[illegible] (3)
Other	1 (2 5)	2 (6)	3 (4)
Totals	39 (100)	35 (100)	74 (100)

¹) adapted from Hubbell et al (1965)
²) percentage of deaths in parentheses

TABLE V

Unexplained death in relation to perinatal mortality and total number of infants

Material	Perinatal mortality Per cent	Unexplained death in relation to perinatal mortality Per cent	Unexplained death in relation to the total number of infants Per cent
Present series 846 infants of diabetic mothers	19 7	9	1 8
Baird et al (1954) 26116 unselected infants from a maternity hospital	3 8	33	1 3
Fairweather et al (1966) 14698 *unselected infants from an English city*	3 8	[illegible]	0 7

betic women The rise in toxaemia in the same period is in good accordance with the increasing number of patients belonging to White's group D and F with vascular complications who were seen during the period This also applies to the increase in fatal congenital malformations, since we have observed a strong positive correlation of congenital malformations with maternal vascular complications (9)

From previously published autopsy series (2 3, 7) and the present material (Table IV), the fact emerges that 50 per cent of neonatal deaths had hyaline membrane disease with pulmonary atelectasis as the sole demonstrable patho-anatomical cause of death In the other half (with and without hyaline membrane present), major causes of death were demonstrated fatal congenital malformation, birth injuries, inflammatory lesions and miscellanous causes

These results underline the familiar fact that the respiratory distress syndrome is still the crux of management of the neonate of diabetic women, and furthermore, that congenital malformation plays a significant role as a cause of death

SUMMARY

Causes of perinatal death were analysed from a clinico-pathological point of view in a consecutive series of 167 infants of diabetic mothers In 60 per cent the perinatal deaths were not unexpected and fully explained In 30 per cent the not unexpected deaths could not be definitely explained, while in about 10 per cent the deaths were unexpected as well as unexplainable The last figure is similar to, or less than figures given for the population at large

Fatal congenital malformations have doubled, while fatal obstetrical complications as a cause of death have decreased to one fifth during the last 10 years During the same period, clinical pyelonephritis and toxaemia have increased, whereas precoma and severe acidosis and the state of being a "neglector" have decreased considerably as causes of death

An autopsy study of causes of death in infants of diabetic mothers, dying neonatally during recent years (1960–1966), was in good accordance with an American investigation Half of the deaths were due to hyaline membrane

disease and other respiratory problems, one fourth were due to congenital malformation and one-fourth to other causes including infection, extreme prematurity and birth injuries

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SUBJECT INDEX

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SUPPLEMENTUM

DIABETES IN S

A clinico-statistical, epidemiolog
of hospital patients and d

By

ALBERT GR…BERG, TAGE LAR…

ACTA MEDICA SCANDINAVICA

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DIABETES IN SWEDEN

A clinico-statistical, epidemiological and genetic study of hospital patients and death certificates

By

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STOCKHOLM 1967

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Foreword

The Medical Research Council of the Swedish Life Offices was founded in 1950 with the objects of promoting medico-actuarial research and supporting scientific studies in fields of medicine concerned with diseases which according to available statistics, have a particular bearing on the economy of the life insurance companies

For several years research grants have been given to basic research of problems connected with diabetes mellitus On the suggestion of Dr Arthur Engel Director General of the National Board of Health and Chairman of the Advisory Board of the Council and Dr Albert Grönberg Physician in Chief at the Department of Internal Medicine of the County Hospital at Vanersborg the Council decided to sponsor a retrospective study of certain Swedish hospital data on diabetes mellitus The planning of this study was performed in collaboration between a great many medical and statistical experts in particular the physicians in-chief at the departments of internal medicine of the investigation hospitals selected Drs Albert Gronberg at Vänersborg Olle Hogeman at Falun, Ian Lundholm at Östersund and Otto Östberg at Vaxjo, and the statisticians Drs Per Olof Berge Jan Jung Tage Larsson and Carl Philipson

For the technical data processing the equipment of the Hansa Insurance Group has kindly been made available under the supervision of Mr Bertil Eklund

The study of the clinical material has been supplemented with a special processing of population statistics on causes of death a parallel investigation by Dr Larsson with regard to cerebrovascular disease has recently been published

Further a discussion of the genetic problems in diabetes mellitus has been included in the present study

It is the hope of the Council that this volume will prove of interest to both clinicians and research workers

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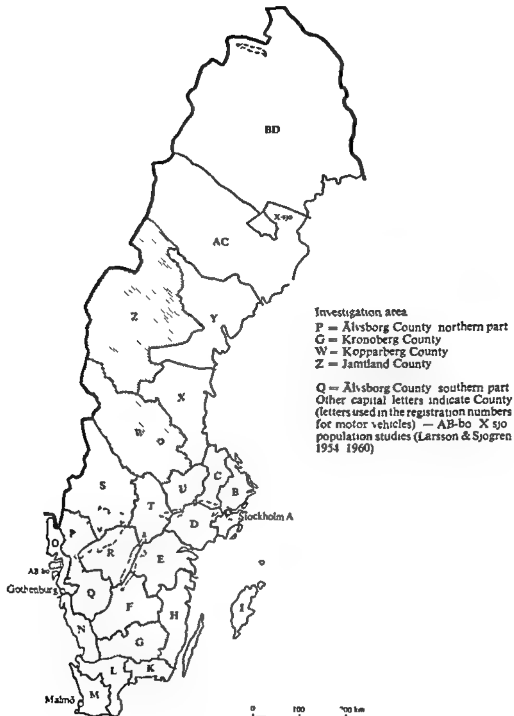


Fig 1 Map of Sweden

Contents

Tables

t

Explanation of terms and symbols

DM = Diabetes mellitus

Grouping by age at onset (O) and duration (D) As a rule the clinical data are grouped by 5 year classes according to age at onset of DM (0–4 5–9 etc) and by 5 year groups according to the duration of DM (0–4 5–9 etc) In order to save space the following notations are sometimes used in respect of groups by duration

a = 0–4	d = 15–19
b = 5–9	e = 20–24
c = 10–14	f = 25–

Age Where the age at a certain event is calculated as the difference between the calendar year of the event and the calendar year of birth the expression *age* is applied Where the exact age is rounded off downwards the expression *attained age* is used If the material has been primarily classified into 5 year groups by age at onset and duration the patient s age has generally been calculated by adding the age at onset and the duration (both in 5 year groups) where this procedure has been applied the expression *average age* is used *Example* age at onset 20–24 (average 22) duration 15–19 (average 17) hence average age 39

Onset type (T) To a great extent it has proved suitable to concentrate the grouping by age at onset into three types viz.

Juvenile onset (J) = age at onset 0–14
Early adult onset (A) = age at onset 15–39
Late onset (L) = age at onset 40–

Sex With a few exceptions all data are given by sex

M = Males
F = Females

Geographical notations County (domicile) is denoted by the capital letter used in registration numbers for motor vehicles (cf Fig 1) For the sake of clarity however Älvsborg County is denoted by PQ (not by the official letter P alone) P is used for the northern part and Q for the southern part

Admission areas of the investigation hospitals

P = Älvsborg County northern part (Fig 2 p 36)
G = Kronoberg County (Fig 3 p 38)
W = Kopparberg County (Fig 4 p 39)
Z = Jämtland County (Fig 5 p 40)

Geographical division of the admission areas (cf Figs 2–5) see Table 11 p 56

Investigation hospitals For convenience and since no misunderstanding is possible the geographical notations have been applied to the four county hospitals covered by the clinical study viz.

P = Vänersborg
G = Växjo
W = Falun
Z = Östersund

Investigation periods

The clinical series in the main 1931–56 (cf Table 1 p 41)
Mortality in the clinical series 1946–55
The population study on mortality (causes of death) 1961–63

Epoch (E) For the analysis of certain time trends the clinical series has been divided into two epochs viz.

E1 *or earlier epoch = duration periods beginning in 1949 or earlier*
E2 or later epoch = duration periods beginning in 1950 or later

Clinical data can concern the presence or absence of a certain complication the maximum value the mean value etc as will be stated in the text

Occasion (occasion card) = the data registered during a certain admission (as inpatient or as outpatient)

Registration (duration card) = the data registered at occasions during a given 5 year duration period

Late complications

Rd = Diabetic retinopathy (Retinopathia diabetica)
Pd = Diabetic nephropathy (Nephropathia diabetica Proteinuria diabetica)
Ud = Diabetic neuropathy (Neuropathia diabetica)

(The letter N has been avoided in this connection instead the third letters have been used viz P and U respectively)

Causes of death Since 1951 the statistics on causes of death in Sweden have been presented in accordance with the W H O scheme *Manual of the international statistical classification of diseases injuries and causes of death* Geneva 1948 and 1949 Certain amendments have been adopted in particular some from 1960 with regard to the delimitation between accidents and disease when stating the primary (underlying) cause of death As a rule the data given here refer to the Intermediate List (the A list) The following notations are used (abbreviations in *italics*)

I	Infective and parasitic diseases (A1–A43)	*Infect*
II	Neoplasms (A44–A60)	*Neopl*
D	Diabetes mellitus (A63 = 260)	*Diab*
VI	Diseases of the nervous system and sense organs (A70–A78)	*Nerv*
C	Cerebrovascular disease (A70 = 330–334)	*Cerebr*
VII	Diseases of the circulatory system (A79–A86)	*Circ*
VIII	Diseases of the respiratory system (A87–A97)	*Resp*

IX	Diseases of the digestive system (A98–A107)	*Digest*
X	Diseases of the genito-urinary system (A108–A114)	*Gen urin*
Oth.	Other diseases (A61–A62 A64–A69, A115–A135)	*Oth dis*
XVI	Symptoms, senility and ill-defined conditions (A136–A137)	*Unknown*
XVII	Accidents poisoning, and violence other than suicide (AE138–AE147 AE149–AE150)	*Accidents*
XVIII	Suicide (AE148)	*Suicide*
Viol.	XVII + XVIII	*Violence*

Unless otherwise stated all statistical data in Chapter IV refer to the period 1961–63

Abbreviations in Chapter IV

P = 3 × average population 1961–63
T = Number of deaths 1961–63
D = Number of deaths from DM 1961–63
C = Number of deaths from cerebrovascular disease 1961–63
T/P = Death rate (as a rule given by sex and age)
D/T = Share of deaths from DM (as a rule given by sex and age)
D/P = Death rate for DM

Area u = urban r = rural

Classification of causes of death (annexed lower-case letter)

p = primary (underlying) cause
c = contributory cause (or complication)
t = total = p + c

Autopsy (annexed lower-case letter)

a = autopsy performed

General remarks

Other abbreviations are explained in the tables in which they are used

Population data for a calendar year refer to the end of the year

In some of the tables exaggerated exactitude has to a certain extent been used

In order to avoid obscurity simple plotting of class data is used in the figures although in many instances histograms would have been appropriate

Currency

All amounts are in Swedish kronor in 1967 the equivalent of 1 000 kronor is about £ 70 sterling or 195 U S dollars.

Symbols

per cent
— magnitude nil
data not available or too small to permit calculation
= category not applicable

Introduction

The present study concerns *clinical diabetes mellitus* (DM) in Sweden

It is a widespread opinion that the incidence of DM has been increasing during later decades possibly for both genetic and environmental reasons It is also widely held that although early deaths from DM can to a great extent be prevented or postponed thanks to insulin therapy the incidence of late complications in the form of retinopathy nephropathy and neuropathy is steadily rising In some quarters it is maintained that insufficient dietetic control has largely contributed to this development

There are three main ways of approaching these important problems

(a) epidemiological population studies in order to evaluate the prevalence of DM (and various DM complications) by sex and age

(b) statistical mortality studies in order to evaluate the significance of DM (and its various complications) as a cause of death hence the excess mortality for diabetics and in order to evaluate the prevalence of DM among deaths by sex and age

(c) clinical studies of DM patients in order to evaluate the course of the disease by time sex age at onset duration and treatment including among other things the incidence of various complications

In some counties in Sweden screening investigations on a large scale have been performed or are in progress, with a view to ascertaining the prevalence (by sex and age) of clinically manifest diabetes and of suspected prediabetes (diabetic glycosuria, hidden diabetes and in addition, renal glycosuria)

In certain university clinics (departments of internal medicine ophthalmology and paediatrics) there have been performed thorough studies of late complications in long standing diabetes and of the special features encountered in child diabetes There have also been a number of investigations of hospital patients dealing with variations by time (hence comparison between different periods) and by treatment (hence comparison between different diets different types of insulin etc) Some of these studies will be reviewed and discussed in the following chapters

As a matter of fact current opinions concerning the morbidity risk and the prevalence of DM in different groups by sex and age and the excess mortality for diabetics by age at onset and duration are not supported by reliable investigations in Sweden but are mainly based on

experience from other countries, especially the U S A With regard to late *complications and the role of diet, etc* the results obtained diverge in many respects and this can, at least in part, be ascribed to a series of selective factors In what follows a fairly large amount of space will be devoted to the analysis of such selective factors, in particular those occurring in retrospective studies of clinical material

The material used in this investigation consists of mortality statistics and hospital records only no screening or other population studies were performed

Since 1951, the W H O classification of causes of death has been applied in Sweden but deaths from DM were also registered in the older classification schemes In fact during a certain period the instructions for the statistical classification contained the rule that if the deceased person had not succumbed from violence (accident, suicide homicide) or cancer and had been afflicted with diabetes diabetes should always be considered the cause of death Although this practice was abandoned many years ago it long had—and still has—the effect that as a general rule doctors making out a death certificate will mention DM—as the primary cause or as a contributory cause—whenever they are aware that the deceased person was afflicted with diabetes Until 1960 the processing of the statistical data on causes of death referred to primary causes alone but since the beginning of 1961 data on contributory causes and complications have been included The material from the years 1961–63, in all 3 324 deaths with DM as the primary cause and 7,273 deaths with DM as a contributory cause, is analysed in Chapter IV[1]

For the study of hospital patients—inpatients as well as outpatients—there were selected, for special reasons, four medium sized general hospitals in different parts of Sweden These are the county hospitals of Vänersborg (in Älvsborg County, P) Växjo (in Kronoberg County, G), Falun (in Kopparberg County, W) and Östersund (in Jämtland County, Z) The registration covers only the departments of internal medicine at these hospitals, hence not the paediatric departments The reasons for selecting these hospitals will be presented in Chapter II In all, this material covers 3,759 patients registered at the hospitals as afflicted with DM, mainly during the period 1931–56, with a total of about 45 200 admissions (or readmissions) and a total of about 19 700 observation years

The data in the case records for these patients were transferred to punched cards (one for each separate admission, *occasion card*) and processed by computers into *duration cards* (one for each 5 year duration period, reckoned from the calendar year of onset of DM) These duration cards, in all 7,385 were in their turn used for the further processing of the data The results of the analysis performed with regard to course and complications are given in Chapters V–VII

For all 3 759 patients it was investi

[1] Recently the Central Bureau of Statistics published detailed data on mortality patterns by primary cause in Sweden in 1961–63 (Bolander 1967)

gated whether they were still living at the end of 1955 or had died before that date and in the latter case, the year of death This information was used for an analysis of the mortality of DM patients during the period 1946–55, covering in all 16 440 observation years and 398 deaths during the period The results of this mortality study are presented in Chapter III

In Chapter I a general discussion of clinical aspects on DM and Swedish practices in the treatment of the disease is given and in Chapter VIII the rating of DM risks in life and health insurance in Sweden is discussed

The results from the analysis in Chapter IV reveal that the prevalence of DM is far greater than has hitherto been considered to be the case The life table expectancy for DM can be assessed at nearly 5 per cent for males and over 10 per cent for females probably the total morbidity risk for DM is about 6 5 per cent for males and about 13 per cent for females The implications of these findings for the theories of the genetic aetiology of DM are analysed in Chapter IX

The general planning and scope of the investigation statistical aspects on the projection adaptation and judgment of clinical research and certain preliminary results of the clinical study have been reported earlier (Grönberg 1962 Larsson 1962 Jung 1962)

CHAPTER I

General aspects on diabetes mellitus and treatment practices in Sweden

As mentioned in the Introduction the present study is mainly concerned with two problems viz the symptoms and course of clinically diagnosed DM and the incidence and prevalence of clinically (or patho-anatomically) diagnosed DM in Sweden The study of the first of these problems is based on all patients admitted to the departments of internal medicine at certain county hospitals during periods beginning around 1930 and ending around 1956 (inpatients as well as outpatients) The study of the second problem is based on statistics concerning causes of death for the period 1961–63 (primary causes as well as contributory causes)

Of Swedish studies dealing with child diabetes may in the present connection in particular be mentioned those by Lichtenstein (1938 1939 1949 1949) Lichtenstein Y Larsson and Ploman (1952) Engleson (1954) Kornerup and Ström (1958) Y Larsson (1962) Y Larsson and Sterky (1962) Bergstrand Furst Y Larsson and Sterky (1962) and Sterky (1963)

As a background certain data on the organization of health services in Sweden will be given and some general aspects concerning the treatment practices in Sweden in respect of DM will be discussed

The organization of health services in Sweden

With regard to the organization of the Swedish health services, it should be mentioned that all activities in the field of public health and medical care in Sweden are either operated or supervised by public authorities The *National Board of Health* is the principal instrument of the state for governing superintending and promoting the activities and the work of the institutions pertaining to this field The main part of the administration of the somatic health services rests with local authorities The county councils 25 in number, or in six of the largest cities the borough councils are responsible for seeing that the sick receive the hospital care required in addition within their field of responsibility fall the organizations concerning district public health nurses district midwives public dental care etc Until now, the state has administered the organization concerning the district medical officers (who receive a certain salary are entitled to a state pension and have to follow a special tariff in charging fees)

At the end of 1930 when the population of Sweden was 6 14 million there

were 2 376 authorized physicians, of whom 1 448 (including the district medical officers) were working in public service 791 were private practitioners and 137 had retired At the end of 1956, when the population was 7 34 million, the number of authorized physicians had increased to 6 045, of whom about 300 had retired From an international angle, the number of doctors may appear to be very low, but it should be pointed out that the number of qualified nurses and other medical personnel is large In 1956 there were more than 18,000 qualified nurses and the other medical personnel numbered over 83 000 (not including administrative and domestic staff at hospitals)

At the end of 1956, the number of beds in hospitals was about 107,500 of which 63 700 were in somatic hospitals (43,800 in mental hospitals and institutions for oligophrenics and epileptics) Of these 63,700 beds all but 1,600 were operated by public authorities In 1956 there were 910 000 admissions of inpatients at somatic hospitals (including homes for the chronically diseased) with a total of 18 86 million bed days These figures mean that on average 51,500 inpatients a day were treated in somatic hospitals At general hospitals proper (with 34,500 beds) the number of inpatients a day was 28 700 On average these latter hospitals were visited on each working day by 14 500 outpatients (not including patients at X ray departments) who were treated at polyclinics organized either by the authority responsible for the inpatients care or by the physicians in accordance with an agreement between them and the authority, the first mentioned system applies to the four county hospitals included in the present study

Under the Swedish social security system health insurance has been compulsory since 1955 As a rule this insurance covers all fees charged for treatment in the public ward of a hospital three quarters of the fees for medical treatment of outpatients and the doctor s fees for treatment of patients outside the hospitals (limited however to three quarters of the amounts shown in the special tariff mentioned earlier) and the patient s necessary travel expenses (above 4 or 5 Swedish kronor) In addition this insurance covers loss of income during periods of illness (according to the rules introduced in 1955 about two thirds of the daily earnings corresponding to income up to 22 000 kronor a year, except in respect of the first three days, as from 1967 the coverage has been increased) To a great extent, these benefits are supplemented by means of private insurance (cf Alvarson 1966)

For medicines prescribed by a doctor the insurance covers 50 per cent of any cost exceeding 3 kronor Certain medicines among them insulin, are free of charge

Prior to 1955 health insurance was not compulsory but the majority of the active population had arranged for a similar coverage in respect of hospital care (where the fees charged for treatment in the public ward were very low anyhow) other medical treatment, and a certain although in general modest sum to compensate for loss of income

The international economic crisis of the early thirties affected Sweden less

than many other developed countries and during the last 30 years employment has been high The standard of living has greatly improved, especially during the last few decades

It may be said by way of summary that during the period studied there were no substantial economic obstacles to the obtaining of medical care in hospitals or from physicians

Treatment practices in respect of DM

With regard to the pre insulin era it may suffice to mention that dietetic regulations were ordinarily used in uncomplicated cases of maturity-onset DM As a rule it was considered satisfactory when glucose in the urine was below 1 per cent[1] Little attention was paid to the existence of acidosis The diagnosis was based on anamnestic data and the presence of glycosuria Blood sugar tests were not generally applied until the middle of the 1920s It may further be remarked that patients with maturity onset DM often sought medical aid at a comparatively late stage of the disease, a common reason for doing so was the occurrence of carbuncles or threatening gangrene

Since 1923 insulin treatment has been generally available in Swedish hospitals Thus at the beginning of the period covered by our hospital series insulin therapy had been used for about seven years and Swedish doctors were familiar with the efficacy of the new remedy and with the techniques of the treatment The results had been excellent It is therefore not surprising that most authorities were convinced that the main problems concerning the cause of DM and its treatment had been solved The cause was a deficiency of insulin and the therapy should be administration of insulin at least for patients who had presented persisting glycosuria or hyperglycaemia In many quarters the pessimism that—before the discovery of insulin—had prevailed with regard to the prognosis of DM was transformed during the twenties into an almost unbounded optimism The high degree of success obtained even in rather severe cases with early onset of DM, was considered to justify this attitude, it should be remembered that at that time there was a very poor knowledge of the late course of juvenile DM

The most impressive results of the insulin treatment were those observed in the management of acute complications such as ketosis and diabetic coma

The type of insulin available in 1930 was what we now term regular insulin Although this insulin was of good quality it had not the same degree of purity as has the insulin of today

During the early thirties there appeared in the literature papers concerning renal damage and eye diseases which were considered to be connected with DM In the patho-anatomical field great attention was paid to the clearly recognizable renal syndrome described by Kimmelstiel & Wilson (1936) From the social point of view interest was focused on the retinal changes—diabetic retinopathy—with its often very severe consequences in the form of reduced vision or

[1] For convenience this expression 1 per cent is used to denote 1 g sugar per 100 cc of urine

blindness Whereas nephropathy and retinopathy were much discussed the occurrence of diabetic neuropathy does not seem to have been closely studied during this period

A valuable addition to the insulin therapy had been obtained in the middle of the thirties when Hagedorn and his co-workers (1936) succeeded in preparing protamine insulin and protamine zinc insulin These are more slowly dissolved and absorbed than in regular insulin, they act longer and may keep the blood sugar fairly normal for 24 hours (maximum duration 34–36 hours) whereas the maximum duration of regular insulin is 6–8 hours only By introducing long acting insulin it was hoped that the insulin treatment could be reduced to one injection a day To a certain extent this proved possible although many physicians held the view that one insulin injection daily did not give sufficiently good control of the disease

The diets that had been evolved during the pre insulin period still played a predominating role at the beginning of the thirties It proved very difficult to decide what position to take up concerning the diet for patients receiving insulin treatment The prevailing attitude was that the carbohydrate intake should be restricted so much that the glycosuria was eliminated or at least reduced to a very low amount However because a considerable reduction in the supply of carbohydrates gave rise to acidosis it was not possible to carry these restrictions beyond a certain limit At the same time it was necessary that nutrition should be sufficient to keep the body weight at an acceptable level The reduction of carbohydrates was therefore largely compensated for by means of an increased supply of fat

In the literature from this period there are often references to the oat days recommended by von Noorden (1917) together with a drastic reduction of bread and other carbohydrates the patient was prescribed oatmeal porridge and other oat products on certain days in order to prevent the occurrence of acidosis The diets recommended by Allen and by Joslin represent different technical procedures with the purpose of eliminating or reducing glycosuria and avoiding acidosis Allen emphasized the importance of a low fat intake he considered a high fat diet dangerous fat being the mother substance of the ketone bodies Fasting days were included in both these treatments

In Sweden Karl Petren (Professor of Internal Medicine at the University of Lund) had suggested a diet that was widely used during the years preceding the discovery of insulin [1] His prescriptions included a marked restriction of carbohydrate intake however a number of vegetables containing carbohydrates were allowed in free quantities In practice it was white cabbage to which this unrestricted consumption came to apply The most prominent difference in comparison with other diets was that Petren permitted his patients a large intake of fat he did not consider a daily consumption of 300 grammes of fat to be detrimental for in his opinion such an intake would not be accompanied by any increased production of ketone bodies In Petren s treatment there were included

[1] Cf Wilder 1955

fat and vegetable days during which the patient was forbidden to eat other carbohydrates than those present in the vegetables It is noteworthy that a great many diabetics kept their disease under good control with this diet which differs so radically from what is nowadays considered to be the most suitable regimen for diabetics The report of Petrén (1923) concerning his wide experience from the treatment of diabetic patients appeared at the time when insulin therapy began its triumphal progress all over the world

The question of the most suitable amount of carbohydrates to include in the diabetes diet has been much discussed At the Scandinavian Congress of Internal Medicine in Oslo in 1931 Norwegian experts argued in favour of an increase of the daily intake from about 100 grammes, which was at that time the most common recommendation to 250 grammes or more Bang (1929) had found that urobilinuria which was considered to be due to a disturbed liver function was increased when the supply of carbohydrates was reduced so much that acidosis occurred Bang and several other speakers at the congress held the view that an insufficient intake of carbohydrates could give rise to liver damages through hunger acidosis Many have opposed this view but today Bang can be considered to have been right although he exaggerated the risks of liver damage arising in this way

In Sweden there were in the 1930s several advocates of a free diet among them Söderling (1935) Lichtenstein (1938 1939) and Mollerström (1939) As Professor of Paediatrics at Karolinska Institutet Lichtenstein had often met with the great difficulties connected with the administration to children of a strictly regulated diet In his opinion it was more important to keep the child in mental and psychological balance than it was to eliminate glycosuria and to keep blood sugar at the normal level Möllerstrom (1939) considered that a sufficient administration of insulin could as a rule be arranged resulting in a good state for the diabetic without any restrictions in respect of his usual food During the thirties there were considerable divergences concerning the suitability or unsuitability of the free diet

Quite naturally, the discussion with regard to dietary regulations was more moderate in respect of maturity onset DM than it was in respect of juvenile DM Certainly it was maintained that for the former category a well regulated diet could help to prevent or postpone the occurrence of gangrene, and that with such a diet neck carbuncles would not occur or would take a less malignant course but these questions did not engage the protagonists in the same way as did the divergent opinions concerning the appropriate treatment of juvenile diabetics As a rule the doctor was satisfied if the control of maturity-onset diabetes resulted in a 24-hour urinary excretion of glucose that did not exceed 1 per cent It should be remembered that in those days no sulpha preparations or antibiotics were available infections of various kinds were important factors influencing the mortality among diabetics

During the Second World War the supply of food was scarce and the most important foodstuffs were rationed On the basis of observations of the inci-

dence of new cases of DM and the mortality from diabetes during this experiment in nutritional physiology it was argued that a reduced intake of food would prevent or postpone the onset of DM and would have a favourable effect in respect of the course of the disease There were also adduced data from the First World War, for instance from Germany and Finland which were interpreted as indicating a reduced incidence of new cases and a lower mortality from diabetes during this period Although the bearing of these statistics must be regarded as rather limited, there is no doubt that they led to greater interest being taken in the weight problems as regards diabetics [1] The opinion that one of the objectives in the treatment of DM should be to combat overweight is nowadays generally accepted and the correctness of this view is borne out both by physiological experiments and by clinical observations

At the end of our registration period viz around 1956 the methods of treatment had been largely stabilized The general view was that the diabetic diet should be adequate from the viewpoint of nutritional physiology but low in calories There was unanimity that overweight should be combated As a consequence of this attitude towards overweight particular interest was given to the fat intake In certain quarters it was held that this should be low and should preferably consist of polyunsaturated fatty acids, it was considered that this would bring about a favourable effect by reducing the cholesterol content in the blood This in turn would have an advantageous effect in regard to the diabetic vascular lesions The so called late complications—retinopathy, nephropathy, and neuropathy—were the object of intense study In 1956 the general view was that these late complications were real consequences of DM and were mainly ascribable to an unsatisfactory control of the disease

The general opinion today (1967) is that retinopathy and nephropathy are assignable to a vascular change—diabetic microangiopathy Patho anatomically, this lesion is characteristic, and it is regarded as specific for DM During the last years it has been found that the presence of microangiopathy can already be established before the clinical picture of DM has developed By this it is proved that the so-called late complications from *the kidneys and the eyes are not to be* interpreted as a consequence of clinical diabetes with a long duration but are vascular changes which run their course parallel with the clinical disease It is still an open question whether the treatment and control of DM has any influence on the occurrence and course of the late complications mentioned It is obvious, however, that from the statistical point of view there is a correlation between the duration of DM on the one hand and the prevalence and severity of the vascular changes on the other, in evaluating this correlation due account should of course be taken of mortality and other selective factors

When giving advice to their diabetic patients Swedish doctors—like their colleagues in other countries—generally emphasize the importance of regular

[1] Cf I Vartiainen and O Vartiainen (1944) Saltzman (1945) and I Vartiainen (1947)

habits and the salutary effect of proper exercise In these respects no changes in practice occurred during the period studied but of course there must have been individual differences connected with the character of the examinations and the length of the treatment, the symptoms and course of the disease, the patient s occupation and working capacity, as well as with the doctor s temperament and general attitudes

The diagnosis of DM

Around the end of our registration period, Elliott P Joslin (1953) applied the following definition of DM Diabetes is an hereditary disease in which there is an increase of sugar in the blood and excretion of sugar in the urine it is dependent upon disease of the pancreas, particularly of groups of cells called the islands of Langerhans which have close connection with several other glands in the body the secretion of the islands of Langerhans—insulin—not only promotes the normal storage of glycogen (animal starch) in the liver, muscles and skin and the combustion of glucose (sugar) in the tissues but also exerts a control upon the transformations of protein and fat Thus at that time the conceptions concerning the mechanism of DM were largely the same as those prevailing in the twenties

As stated already our series of DM patients consists of persons admitted to the departments of internal medicine at four county hospitals After the establishment of separate paediatric departments children afflicted with DM are not included unless they have attained an age of about 15 years, these patients will then as a rule be taken over by the departments of internal medicine

In respect of juvenile diabetes the on set of DM is generally connected with marked acute symptoms In respect of adults the onset is less alarming and often insidious but more or less typical symptoms—in the form of thirst, hunger, emaciation loss of weight, weakness frequent urination itching boils or carbuncles, 'sweet breath odour, etc —will often cause the patient to seek medical advice (either as an outpatient at a hospital or by consulting a physician outside the hospital) In developed cases, the symptoms are in general sufficiently clear to be interpreted as being due to DM however since for patients with symptoms of the kind mentioned urine examination is routine the doctor will base his preliminary diagnosis on the occurrence of glycosuria Unless this glycosuria can be eliminated by means of simple dietary regulation, the patient will as a rule be remitted to a hospital for further investigation in particular by means of blood sugar tests

Through the routine urine examination the occurrence of glycosuria will often be observed when a patient is seeking medical advice for reasons other than subjective symptoms indicative of DM This applies not only to the clientele of doctors outside the hospitals but also for instance to patients in surgical and gynecological departments at hospitals In some cases an eye examination may reveal retinal changes which are interpreted as indicative of DM and the patient will be remitted to the depart-

ment of internal medicine for further examination

The definitive clinical diagnosis of DM is primarily based on the occurrence of glycosuria and hyperglycaemia All patients included in our hospital series have presented both glycosuria and hyperglycaemia

During the whole registration period a positive test of glycosuria (i e the presence in the urine of a reducing substance) has been considered indicative of DM

Hyperglycaemia has been considered to exist where the fasting blood sugar (i e the blood sugar after at least 10 hours of fasting) is 0 11 per cent or more [1] Stress has been laid on the requirement that the patient shall have eaten a sufficient amount of carbohydrates during the days preceding the test

In the early twenties there were elaborated comparatively easy and reliable methods for serial determinations of blood sugar, and glucose tolerance tests (GTT) were brought into use in the diagnosis of DM At the hospitals here studied glucose tolerance tests—oral intravenous or both—have been applied where the diagnosis has not already been established on the basis of the fasting blood sugar In the case of normal fasting blood sugar and negative test of glycosuria glucose tolerance tests have as a rule been performed only if there is information (from a doctor or from the patient himself) about sugar in the urine on earlier occasions A fairly common experience has been a positive oral test and a negative intravenous one, but sometimes the opposite has applied In general a clearly normal outcome of the oral tests has been considered to exclude the diagnosis DM

The techniques used in respect of the glucose tolerance tests will be commented upon in a later section (cf p 122) It may suffice to mention in this connection that the evaluation has been based in particular on the 2 hour value

In respect of the diagnosis of DM among patients admitted to a hospital there are only a few alternative diagnoses to take into consideration

Glucose will be found in the urine when the blood sugar level exceeds the renal threshold However there are individual variations and also variations with age A young diabetic will often present glycosuria with a blood sugar of 0 13 to 0 15 per cent whereas an elderly diabetic may have a blood sugar level of 0 18 per cent or more without showing glycosuria It is often technically difficult to arrive at a fair degree of certainty concerning the renal threshold In *renal glycosuria* this threshold is lowered and then glycosuria is persistent even if there is no hyperglycaemia Persons with renal glycosuria are often remitted to a hospital for investigation especially if they are seeking a health certificate (in connection with an application for a driving licence life insurance etc) or where glycosuria has been observed at a routine urine examination by the patient s own physician As already mentioned in our series patients without hyperglycaemia are not included

General or specific *starvation* may give rise to ketosis Since ketonuria is one of the first evidences of incipient DM, there

[1] For convenience this expression 0 11 per cent is used to denote 110 mg sugar per 100 cc blood

may sometimes be a risk of overdiagnosing In the case of acute infections, children may present acetonuria often in combination with sweet breath, head ache nausea and vomiting Old people who do not eat a sufficient amount of carbohydrates and proteins may develop acidosis and acidosis may appear as a sequela of nephritis and certain other diseases Often in these cases a glucose tolerance test made immediately will give a result recalling diabetes However when carbohydrates are administered to the patient during several days (200 grammes or more daily) the glucose tolerance test will show a normal picture As already mentioned it has been a routine procedure at the hospitals studied to arrange that in uncertain cases the patient shall receive an ample supply of carbohydrates during three days before the determination of the fasting blood sugar in this way the majority of the potential in stances of starvation without diabetes will be eliminated

Alimentary glycosuria (accidental glycosuria) caused for instance by the in take of large amounts of sugar may be observed As a rule these patients are treated by simple dietetic regulation viz. no sugar or sweets In general as a safety measure they have been put under surveillance although with progressively longer intervals between the control examinations (increasing to six months or more)

What is sometimes called *pregnancy glycosuria* can be regarded as an expres sion of a temporary overproduction of growth hormones In such cases the blood sugar level is in general normal but slight hyperglycaemia may sometimes occur As a rule the presence of gly cosuria is established at the routine examinations of the maternity welfare services The diabetogenic effects of growth hormones are currently the object of intensive research, but in preg nancy glycosuria the glucose tolerance tests will in general show normal values (cf Hagbard 1956)

Disturbances of the hormone balance connected with the *climacteric* may act as precipitating factors for the onset of DM In respect of these patients the diagnostic criteria and the treatment procedures have not deviated from those applied to other DM patients

Mental and psychological stress may precipitate acute diabetic symptoms, sometimes to a very marked degree and a clear remission will often occur In these cases, the normal diagnostic criteria are applied but restrictivity is observed in respect of the treatment—the primary prescriptions being dietetic regulation and if appropriate sedatives Insulin therapy is regularly postponed until the circumstances are considered to have become stabilized

Cushing s disease (adrenal cortical hyperfunction) may cause glycosuria hyperglycaemia and hypertonia often to a very marked degree In this disease the diabetic signs are to be regarded as symptoms of the underlying disease In gen eral the patients are admitted because of hypertonia Diagnostic criteria are among others obesity moon face , increased excretion of 17-ketosteroids in the urine and disturbances of the electrolytic balance The treatment is that of the underlying disease (as a rule epinephrec tomy)

Starvation cases in need of closer observation as to the possibility of DM have been comparatively rare The same applies to alimentary glycosuria Cushing s disease is seldom found Certain other states which may cause diabetes-like symptoms for instance physical traumata poisoning and pancreatic cancer, have not given rise to any diagnostic difficulties in the present series Among patients seeking medical advice because of disturbances of the carbohydrate metabolism glycosuria and subjective diabetic symptoms, the frequency of renal glycosuria may be assessed at about 2 per cent Hence the quantitative significance of the problems of differential diagnostics is comparatively small

With regard to the differential diagnosis it may be added that in children *with an acute onset of clearly diabetic* symptoms there may sometimes occur a remission with normal blood sugar values even in glucose tolerance tests this remission may last for some months exceptionally for a year or more It has been a routine procedure during the whole period studied that these children are kept under close observation

In the literature on diabetes one often meets with the expression *prediabetes* There are few problems that have given rise to such intense discussions as the *concept and definition of prediabetes* Goldner (1962) is of opinion that this concept should be eliminated if it is intended to cover those situations where a slight disturbance of the carbohydrate metabolism can be ascertained but is not of such a degree as to warrant the clinical diagnosis DM Goldner holds the view that all these cases should be classified as DM Extensive research during the last few years has led to a modified definition it is argued that the diagnosis prediabetes should be reserved for cases without disturbed carbohydrate metabolism but with signs indicative of a probable onset of DM earlier or later The signs in question are vascular symptoms in the form of microangiopathy, and the occurrence in the blood of insulin affinitive substances ('factors), for instance synalbumin In certain quarters it is held that a woman who gives birth to a child weighing 4 5 kg or more belongs to the group of prediabetics Prediabetes under the concepts mentioned in this paragraph is not included in our series [1]

The hospital series studied

As will have appeared from this short survey, the admissions to the hospitals studied cannot be regarded as wholly representative of the total population of diabetics in Sweden In the areas covered by the hospitals there will be a number of diabetic patients who are not admitted because a doctor regards it as appropriate to keep the patient in a sufficiently good state by means of dietetic regulation and general advice alone Of course, there are in addition a number of persons with *a clinically manifest diabetes who do not* seek medical advice at all—at least during the initial period of the disease—and for whom the diagnosis will be made only if

[1] According to the terminology recommended by the World Health Organization (W H O) *prediabetes* should be used in retrospect when reviewing a case to describe the period of time from conception to the diagnosis of an episode of DM

they seek a doctor or are admitted to a hospital because of intercurrent disease

Further, owing to geographical factors, communications etc, the extent to which a county hospital covers different parts of its admission area will vary In the distant parts of this area, the patients may find it more convenient to go to another county hospital that is closer to their homes or is more easily accessible and they may to some extent prefer to go to a local hospital The biases arising from these circumstances will be discussed in a later connection however it should be emphasized that in the main the patients admitted to the county hospitals studied can be regarded as fairly representative of persons who show DM to a degree that—where thorough clinical examination is performed—will be classified as an instance of clinically manifest DM

The material for our study of clinical DM derives from a period when the therapy was based solely on dietary control general health measures and insulin The oral remedies that came into use after 1955 were either not applied at all or applied only (to a small extent) during the latter part of the registration period However because oral medicines nowadays play a not inconsiderable role in the treatment of DM they will be taken up for discussion in a later connection (cf p 164)

Whereas during the registration period the diagnostic procedures were practically the same at each of the four hospitals studied (as well as in most other Swedish hospitals) there were certain differences with regard to the treatment practices and the scope of the control examinations both in time and between the hospitals In particular, the attention paid to the investigation (and registration) of late complications presents some clear discrepancies, which will be analysed in detail in a later section So far as treatment is concerned the most relevant differences apply to the intervals between two consecutive control examinations However it should be emphasized that this type of differences is primarily (though rather fortuitously) connected not with the hospitals *per se* and the views of the doctors but with such external factors as the distance from the patient s home to the hospital the patient s occupation, communications and so on

Where a definite diagnosis of DM had been made the treatment was to a great extent determined by the occurrence or absence of acidosis Patients showing acidosis were regularly hospitalized (viz taken as inpatients) for thorough scrutiny and adaptation if acidosis could not be eliminated insulin treatment was as a rule introduced On the other hand if there was no acidosis attempts were generally made to arrive at satisfactory control by means of increased dietetic regulation Often this was done chiefly for psychological reasons the patient must become convinced of the advantage of dietary restrictions Insulin therapy should not be resorted to on weak indications if later on insulin is deemed appropriate the ground must have been prepared As a rule these patients were treated as outpatients with renewed control after a week or a fortnight If the control examination did not reveal glycosuria or acidosis the dietary treatment

was carried on, with renewed control every month or, later, with an interval from two to three months When indicated the patient was taught to perform urinary tests himself If a control examination revealed the presence of acidosis, the patient was hospitalized for ten days or a fortnight, as a rule insulin treatment proved to be indicated Nowadays with persistent glycosuria but in the absence of acidosis, recourse is generally had to oral treatment (sulfonylureas) during the registration period, there may have existed certain differences with regard to these cases, viz as to whether the most suitable treatment for a certain patient would be continued attempts with dietetic regulation or the introduction of insulin therapy (cf for instance Gronberg 1961, 1963)

Classification by age at onset

There is no unambiguous parameter by which the various types of DM can be classified In the present study we have chosen to group the patients by age at onset of DM This procedure has been deemed to give the most suitable basis for a non selective division in respect of the general clinical picture and course of DM One group consists of patients who had become afflicted before the age of 15 this group comprises instances of juvenile onset diabetes alone (*juvenile diabetes*) One group consists of patients who had become afflicted at an age of 40 or more this group can be regarded as comprising instances of maturity onset diabetes alone (*late diabetes*) Finally, an intermediate group consists of patients who fell ill in the age interval 15–39, in this group there are both patients who would have been classified by other investigators as instances of juvenile diabetes and patients who may be regarded as instances of late diabetes however since a division into only two groups would have entailed certain disadvantages and might be said to overemphasize the significance of the age of onset we preferred to work with such an intermediate group (termed, in the absence of a more appropriate designation, *early adult diabetes*) Where a finer division is called for, the patients are grouped into 5 year classes by age of onset (0–4, 5–9 etc)[1]

The clinical characteristics of the type of DM ordinarily seen in youth and adolescence differ in many respects from those found in adult patients especially if the onset has taken place in middle age or at an advanced age In particular there is a marked difference as regards the liability to ketosis the DM of youth and adolescence being ketosis prone and the DM of the elderly being ' ketosis-resistant

In 1956 Bertram put forward the theory that a proper classification of DM could be made on the basis of the patient's tendency to obesity (cf Bertram Bendfeldt & Otto 1956) The lean type which is often found among young diabetics was termed by him Insulinmangeldiabetes' , whereas the fat type was termed Gegenregulationsdiabetes However, Bertram s theory is not supported by later studies concerning the mechanism of the insulin secretion

[1] Concerning the definition of the expressions age attained age and average age cf p 12

Some factors of importance for the development and course of DM

As already stressed exercise is an important factor in the treatment of DM On the medal instituted by Elliott P Joslin in 1947—the Quarter Century Victory Medal for Health which is given to patients who after 25 years duration of DM are free from late complications—there is the inscription "INSULIN EXERCISE DIET"[1] Joslin wanted to emphasize that in his view these factors were basic in the treatment of DM and particularly with regard to the prognosis of the disease He always advised his patients to practise permanently and regularly some kind of strenuous muscular activity He was convinced that muscular effort brought about increased combustion in particular of carbohydrates and thus in every respect an ameliorated metabolic situation

During recent years experience has in different ways provided evidence that Joslin was right on this point His idea was that the muscular activity directly caused an increased oxidation of glucose in the muscle cells and in consequence a reduced blood sugar level It is certain however that the actual situation is far more complicated Goldstein (1965) is of opinion that the muscular tissues produce what he terms a muscular activity factor which causes a reduction of the blood sugar level this would at least in part explain the beneficial effect on DM of hard physical work A doctor who has had the opportunity of surveying a large number of diabetics over a long period cannot possibly remain unaware that heavy work often has very advantageous effects on the actual state of health of a great many of his patients It is possible that for these physically hardworking diabetics the prognosis with regard to late complications is better than for other patients, but it is difficult to arrive at definite statistical evidence in this respect

In 1889, von Mering and Minkowski were able to prove the existence of a connection between the carbohydrate metabolism and the cell islands in the pancreas that had been detected by Langerhans in 1869 In 1921 came the discovery of insulin A considerable proportion of the research activity in the DM sphere is still devoted to investigations concerning the formation of insulin its secretion and its physiological actions

For many years clinicians biochemists, physiologists histologists and pathologists have been engaged in theoretical and experimental research projects dealing with different aspects of DM A comprehensive picture of the present activity in these fields in Sweden is given in the papers from the Wenner Gren Center Third International Symposium (Brolin Hellman & Knutson 1964)

Vascular changes of different kinds have long interested diabetologists In this field research has been focused on the connections between arteriosclerosis and DM as well as on the specific diabetic microangiopathy It is still an open question whether DM really promotes

[1] The medal is awarded by the Diabetic Fund of the Boston Safe Deposit and Trust Company upon recommendation of its Advisory Committee Cf Elliott P Joslin *Diabetic Manual for the Doctor and Patient* 9th ed 1953 pp 215-217 —We find it noteworthy that Joslin put Exercise next to Insulin but ahead of Diet

the occurrence of arteriosclerotic changes Several authorities claim that diabetics are considerably more exposed to cardiac infarctions than are comparable non-diabetics The appearance of gangrene in the toes has long been considered ascribable to DM however no definite proof of the assumed causality seems to exist The general opinion of today is that the arteriosclerosis found in diabetics does not differ patho anatomically from the arteriosclerosis of non diabetics

There is an abundant literature concerning the significance of atheromatous changes, hypercholesterolaemia and hyperlipaemia in DM patients However, in our hospital series the registration in the case records is not sufficiently informative, and therefore the problems connected with atherosclerosis blood cholesterol level and blood lipid values will not be taken up for analysis

Since the electron microscope became available the study of diabetic microangiopathy has attracted great interest Bloodworth Jr (1963) showed that the vascular changes that are found in the smallest vessels of the kidneys the eyes and the skin are in principle the same The essential change is a thickening of the capillary basement membrane This change has been considered to be so specific that a measurement of the thickness of the membrane would be decisive for making the diagnosis DM Levine (1964) regarded the analysis of a small biopsy from the lobule of the ear to be sufficient for determining whether or not the patient had prediabetes However, according to Lundbæk (1965) the measurement of the thickness of the basement membrane is far more complicated than was thought earlier Bloodworth Jr (1965) has in some respects corrected his interpretation from 1963 However, although adequate routine procedures are not yet available, it seems beyond doubt that electron microscope techniques will be helpful in the diagnosis of DM at an earlier stage than has hitherto been possible

Over a long period attempts have been made to explain the neurological changes often observed in elderly diabetics as caused by vascular lesions of the peripheral nerves (Fagerberg 1959) definite proof of the correctness of this hypothesis is still lacking Through investigations by Olsson Säve Soderbergh Angervall and Sourander (1967) it has been shown that juvenile diabetics too present spinal and cerebral changes which can hardly be attributed to vascular lesions either of arteriosclerotic origin or connected with microangiopathy

According to several investigations it is not uncommon that in the case of ulceration of the feet diabetics show grave signs of diabetic neuropathy but only slight circulatory disturbances ascribable to vascular lesions Further it is common that such trophic ulcers are accompanied by skeletal changes (Gronberg 1966 1966a) This syndrome is known as diabetic osteopathy it has been dealt with by Bailey and Root (1947) Azerad, Lubetzki, Stuhl and Slotine (1961) G Bergqvist (1963) and others Studies are in progress with the purpose of elucidating the cause of these changes Probably there is a disturbance in the function of the autonomic nervous system

Until some years ago it was the generally accepted view that DM is a disease

which is due to the incapability of the body cells to transform carbohydrates and is therefore especially expressed in the form of glycosuria Nowadays fat and fat metabolism are considered to be more important than was assumed earlier The fatty tissue was supposed to be a means of storing reserve energy, this store could be drawn upon according to need but otherwise it was thought to be inactive Investigations performed in recent years have revealed however, that the fatty tissue is highly active and that for this activity it requires comparatively large amounts of insulin Swedish research workers have made interesting contributions in this field Svanborg (1965) has investigated the role of fatty acids in the circulation of fat Björntorp (1965) has analysed the fat metabolism within the body cells and Östman (1965) has arrived at valuable results with regard to the endogenous fatty acid metabolism in diabetics

The mechanism of the development of DM is investigated from several aspects and by different methods

Luft and co workers have devoted much attention to the role of the hypophysis for the occurrence of diabetes (Luft 1965 1965 1965, Luft & Cerasi 1964 Ikkos & Luft 1960 1962 Almqvist Hall Lindstedt Lindsten Luft & Sjoberg 1964 Ikkos Luft Gemzell & Almqvist 1962) They have shown that the administration of a sufficiently large dose of human growth hormone gives rise to clear diabetes with hyperglycaemia as well as acidosis Earlier studies by Luft Ikkos Gemzell and Olivecrona (1958) have given valuable information concerning the role of the hypophysis for the development of diabetic retinopathy

Levine and Luft (1964) have analysed the relationship between growth and the diabetogenic effects of growth hormone Their theory is that there exist two factors one of which (the real growth hormone) promotes the protein synthesis in the presence of insulin while the other factor (termed the adiposi kinetic factor) is effective in respect of the fat transportation (cf also Forsman & Gemzell 1959)

With regard to the mechanism for the development of DM it should be mentioned that a quite different theory has been set forth by Vallance Owen (1963)[1] In the blood he has found a special factor—termed synalbumin—which according to him is capable of binding insulin and of making it inactive to a certain extent An amount of synalbumin in the blood exceeding 1 25 per cent is regarded by Vallance Owen as a clear sign of subclinical diabetes allowing the definite conclusion that the patient will sooner or later develop manifest DM There have been divergent opinions concerning the validity of Vallance Owen s theory, but that discussion falls outside the scope of the present study, which is concerned with clinical diabetes alone[2]

The significance of genetic factors for the occurrence of DM has been studied and discussed for a very long time Certain problems regarding the hereditary nature of DM will be treated in Chapter IX

[1] Cf also Vallance Owen Dennes & Campbell 1958 Vallance Owen & Ashton 1963 Levine 1964a Vallance Owen 1965 and Arvill Westberg Jonsson Hood & Ahrén 1966

[2] The genetic aspects (cf Vallance Owen 1966) are taken up for discussion in Chapter IX

CHAPTER II

Material for the clinical study of DM

General aspects

Often, an analysis of case records concerning patients afflicted with a certain form of disease covering a sufficiently long period will afford a valuable basis for conclusions with regard to the symptomatology of the disease the results of different types of treatment and the prognostic value of the clinical data However, since in general there are important selective factors of various kinds —for instance, factors connected with diagnostic practice diagnostic accuracy and the completeness of the case records or due to differences by sex age, marital state and domicile in respect of the admission rates and to changes with time of these rates—the material taken up for study has to be chosen with great care, a close scrutiny of the data will as a rule be necessary so that the effects of the biases arising from these differences and changes can be properly evaluated

For several reasons the possibilities of performing such period investigations and of evaluating the selective factors occurring may be considered to be particularly favourable in Sweden not least in respect of diabetes mellitus and other chronic diseases For 150 years Sweden has had the good fortune not to be involved in war and also to have been spared major domestic upheavals The population is very homogeneous both with regard to ethnic origin and religion and from the social point of view The Swedish population statistics have a very long history, and information is to a great extent available for fairly small geographical districts so that it is possible to compare statistical data which concern the investigation series with corresponding data for the general population The official registration of the population is very efficient and reliable the parish-register system is so devised that it is possible to follow internal migration As already mentioned there have not existed any serious economic obstacles to the obtaining of medical care in hospitals or from physicians In the hospitals the case records are as a rule relatively comprehensive and they are easily accessible for research workers (Cf Larsson 1965 Larsson & Sjögren 1954, Sjögren & Larsson 1965)

At the projection adaptation and judgment of medical clinical research there are certain statistical aspects which can be and ought to be taken into consideration It should for instance be borne in mind that the topics studied are not a random choice but an intentional

selection from the total population of possible investigation objects Complications arise owing to selective basic material, disappearances from observation, exclusions and bias, and by time lag at registration and diagnosis Usually, however, the main problem is not how to eliminate selective factors—on the contrary it may often be sound investigation economy to introduce such factors—but how to be able to evaluate their effect

Gradual or sudden changes of the circumstances prevailing in a society—taken in a wide sense—will often give rise to complications which are difficult to survey and may lead to erroneous conclusions Such changes may be social economic or purely demographic (rising standards of living investments in respect of hospital facilities urbanization, industrialization, etc) but they may also be psychological or medical (altered attitudes the use of new drugs in general therapeutic practice new treatment procedures, ameliorated diagnostic techniques, etc) Changed legislation (for instance the introduction of compulsory health insurance from 1955) and exceptional disturbances (war epidemics, etc) may influence medico statistical data—sometimes by introducing a displacement effect (acceleration or delay)

An important factor is that persons who are now old lived in their earlier years in conditions which in several respects were widely different from those applying to the younger age groups of today For instance, there was earlier a high mortality from tuberculosis, for diabetics the excess mortality was much heavier before the discoveries of insulin sulpha preparations and antib otics than it has been during the last two decades Because of these circumstances—but particularly because of the great intensification of internal migration, resulting in an altered pattern for the choice of marriage partner—there has occurred a successive change in the gene composition of the population, with an increased prevalence of heterozygotes and a reduced prevalence of homozygotes in the younger generations (cf Larsson 1965)

In a way, the use of hospital series may be said to present a number of specific difficulties An exceptional or undue inclusion or exclusion of mild cases or severe cases may easily give rise to false conclusions If a registration is made over a certain period, the structure of the material will as a rule be quite different depending on whether the registration covers all patients, admissions or first admissions In respect of many diseases a hospital series does not reflect the prevalence or incidence of the disease in the population Many patients may be cared for by physicians outside the hospitals, and others do not seek medical advice at all The distance to the hospitals their capacity (for instance if there is a scarcity of beds) and the availability of alternatives (general practitioners, specialists outside the hospitals) are also of importance The introduction of new treatment procedures for certain forms of disease may shorten the stay in hospital and this may in turn give room for an increased admission of patients afflicted with another disease, although for this latter form there have been no changes, either in respect of the incidence or in respect of treatment and the length of hospitalization

When investigating a limited geographical area several selective factors may be operating In Sweden generally there has been a flow of migrants from the countryside to urban localities and also from smaller agglomerations to larger ones In the main, this migration comprises the younger groups of working age Very often, the migration tendency for persons afflicted with a certain disease differs markedly from that of other persons of the same age (the general population)

If the investigation covers only part of the country or only a certain type of hospital, it should be taken into account that the diseased persons can apply for hospital care outside the investigation area or go to hospitals of another type For instance, diabetics who are blind or severely disabled may be underrepresented in series from general hospitals because they seek care at special hospitals (schools for the blind, homes, rehabilitation clinics, etc)

Whether or not the diseased persons will be admitted to a hospital may depend in part on the severity of the disease but also partly on such factors as age, sex marital state domicile and communications For instance in respect of diseases which demand bed stay but do not necessitate an operation it is quite usual that a hospital series comprises more married women than married men (even though the prevalence and severity of the disease do not present any sex differences) in respect of mothers with infant children the selective effect may sometimes be the opposite with lower admission rates than for other women or men Further the occurrence of intercurrent disease and psychological traits ('temperament') may play a part obviously, then, for hospital series one should be cautious about drawing conclusions concerning the coincidence of diseases (the correlation of diseases)

Very often there are reasons for taking into account the complications which arise from delayed registration and delayed diagnosing The disease may have begun at a certain point of time but the patient does not come to a hospital until he has had the disease for a certain period (which may vary according to sex, age at onset, etc) Sometimes the diagnosis cannot be made until the disease has gone on for a certain time but then it will often be possible to establish that it began several years earlier It is worth mentioning that statistically speaking the patients are immortal during such a period of delay

Selection of hospitals

The origin of the present clinical study is that one of us (Albert Grönberg) had planned to perform a survey of the patients personally treated by him at the county hospital of Vänersborg during the period 1931–57, in all more than 800 cases of DM However, it was considered highly desirable to cover a larger series of patients and so, on the suggestion of the Director General of the National Board of Health Dr Arthur Engel the registration was extended to comprise three other county hospitals

The population of Älvsborg County in which Vänersborg is situated, was 313 311 in 1930 and 369 679 in 1956 There are

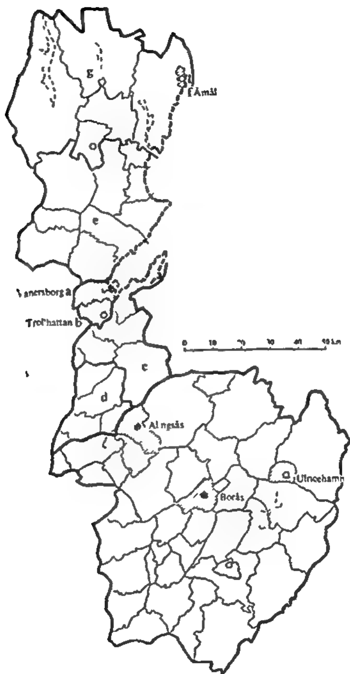


Fig. 2 Map of Älvsborg County (PQ)
P1 (a b) P2 (c d) P3 (e f g) — Cf Table 11 p 56
● general hospital
◘ cottage hospital

three county hospitals (Vanersborg Borås and Alingsås) Vanersborg Hospital covers the northern part of the county with a population of about 130 000 in 1930 and about 145 000 in 1956 The admission area borders on the City of Gothenburg, but it should be noted that Gothenburg belongs to another county (Gothenburg and Bohus County) during the period studied it was a rule (except in instances of a very acute onset of disease) that patients should be treated at a hospital within the county where they had their domicile This applies to out patients as well as to inpatients

In 1930 Vänersborg Hospital had 138 beds with a total of 2 364 admissions of inpatients and 47 900 bed-days In 1956 the corresponding figures were 525 beds with 11 791 admissions and 172 800 bed days of these 525 beds 108 were in the department of internal medicine There had been established separate departments of paediatrics and of ophthalmology in 1947

In addition to the county hospitals of which Borås had 240 beds in 1930 and 603 beds in 1956 and Alingsås had 130 beds in 1930 and 181 beds in 1956 there are some local hospitals (cottage hospitals) and homes for chronically diseased patients in the northern part of the county these local hospitals are Åmal (30 beds in 1930 36 beds in 1956) Bäckefors (32 and 102 beds respectively) and Trollhattan (24 and 39 beds)

In the planning of the present clinical study of DM certain selective factors could be controlled and in the first place this applied to the selection of hospitals Among other things the following criteria could be used

(a) There should be one ' dominating hospital for the relevant parts of the admission area

(b) The physician in chief of the department of internal medicine should be one with great interest in the problems of DM and long experience of the treatment of diabetics

(c) There should not be competition with other hospitals where interest was focused on the treatment of diabetics or with private practitioners specializing in this field

(d) The population should be comparatively stable with a low rate of internal migration over the boundaries of the area, at least in the age groups above 30

(e) The selected hospitals should be suitably spread over the country

Quite clearly Vänersborg Hospital and the northern part of Älvsborg County fulfilled the requirements (a)–(d) Thorough discussions with experts of different kinds and a scrutiny of demographic statistics argued strongly in favour of the further selection of the county hospitals of Växjö in Kronoberg County in the south Falun in Kopparberg County in the middle of Sweden and Östersund in Jämtland County in the north

At Falun Arthur Engel had been head of the department of internal medicine during the period 1943–52 in 1953 he had been succeeded by Dr Olle Hogeman (who was assistant physician in-chief there in 1949–52) At Växjo Dr Otto Östberg had been head of the department of internal medicine since 1939, and both he and several of his pupils had undertaken special research concerning DM (Wallman Carlsson 1950 Engleson 1954) At Östersund Dr Ian Lundholm was physician in-chief of the department of internal medicine from 1930 to 1960 after his retirement the position was held by Dr Leif Thorling from 1960 to 1963

Drs Hogeman, Östberg and Lundholm willingly accepted the proposal

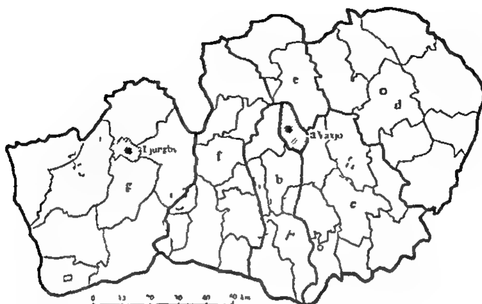


Fig 3 Map of Kronoberg County (G)
G 1 (a) G 2 (b c d e f), G 3 (g) — Cf Table 11 p 56

that their hospitals should be included in the investigation We take the opportunity here to thank them as well as Dr Engel and Dr Thorling for their excellent cooperation valuable suggestions and stimulating discussions

Växjö Hospital covers the greater part of Kronoberg County which had 155 551 inhabitants in 1930 and 159 291 inhabitants in 1956 In 1930 the hospital had 140 beds with a total of 2 213 admissions of inpatients and 52,700 bed-days In 1956 the figures were 374 beds with 10 650 admissions and 129 600 bed-days, of these 374 beds 107 were in the department of internal medicine There had been established separate departments of paediatrics in 1950 and of ophthalmology in 1955

The south western part of Kronoberg County is covered by a non specialized general hospital at Ljungby with 90 beds in 1930 and 108 beds in 1956

In addition to the hospitals at Växjö and Ljungby there are in Kronoberg County two cottage hospitals one at Lenhovda (27 beds in 1930 34 beds in 1956) and the other at Tingsryd (13 and 61 beds respectively)

Falun Hospital covers Kopparberg County with a certain exception for the south eastern part (see next paragraph) The population of Kopparberg County was 249,717 in 1930 and 281 197 in 1956 In 1930, Falun Hospital had 239 beds with a total of 4 696 admissions of inpatients and 102 200 bed-days In 1956 the figures were 496 beds with 13 192 admissions and 150 200 bed days of these 496 beds 122 were in the department of internal medicine There had been established departments of paediatrics in 1943 and of ophthalmology in 1922

The south-eastern part of Kopparberg County is partly covered by hospitals at Avesta (70 beds in 1930 140 beds in 1956) Smedjebacken (38 and 31 beds) and Ludvika (60 and 165 beds) Further in the middle of the county there is a non specialized general hospital at Mora with 80

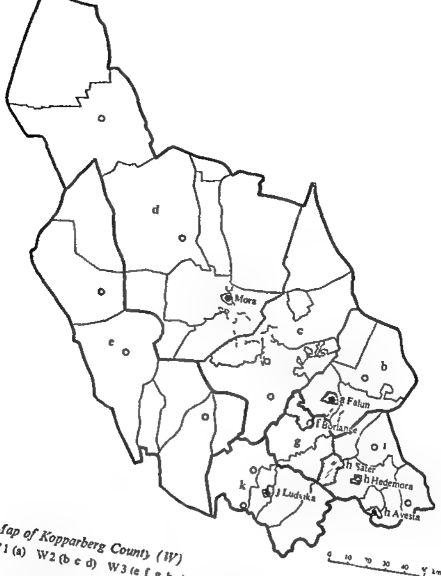


Fig 4 Map of Kopparberg County (W)
W 1 (a) W 2 (b c d) W 3 (e f g h i) W 4 (j k) — Cf Table 11 p 56

beds in 1930 and 103 beds in 1956 There are also in Kopparberg County a number of cottage hospitals with a total of 149 beds in 1930 and 317 beds in 1956

Östersund Hospital covers the whole of Jamtland County with the exception of the south-eastern part (see next paragraph) The population of the county was 134 514 in 1930 and 143 854 in 1956 In 1930 Östersund Hospital had 172 beds with a total of 3 115 admissions of inpatients and 67 700 bed days In 1956 the figures were 375 beds with 10 650 admissions and 132 700 bed days of these 375 beds 96 were in the department of internal medicine There had been established separate departments of paediatrics in 1943 and of ophthalmology in 1940

In the south-eastern part of Jamtland County there is a non specialized general



Fig. 5. Map of Jämtland County (Z)
Z1 (a) Z2 (b c d e f g) Z3 (h) — Cf. Table 11 p. 56

Table 1 Registration periods and annual number of first admissions

Hospital	Registration period	Population of area thousands			Average annual number of first admissions					
		1940	1950	1960	1931–35	1936–40	1941–45	1946–50	1951–55	1956–57
P Vanersborg	1931–57	133	141	149	10	15	22	36	64	56
G Vaxjö	1930–55	151	158	159	4	14	20	54	54	
W Falun	1932–57	248	267	286	6	9	22	56	80	78
Z Östersund	1930–56	139	144	140	14	26	36	57	69	88*
Total		671	710	734	34	64	100	203	267	

* 1956 only

hospital at Sveg it had 72 beds in 1930 and 116 beds in 1956 Further there are in the county two cottage hospitals one at Gadd ede (13 beds in 1930 16 beds in 1956) and the other at Strömsund (22 and 80 beds)

Vänersborg Vaxjo Falun and Öster sund are all county towns (capitals of their respective counties)

Östersund (14 153 inhabitants in 1930 23 790 in 1956) is the only town in Jamtland County In Kronoberg County there is besides Vaxjo (9 699 and 22 381 inhabitants) only the town of Ljungby (4 320 and 8 112 inhabitants) In Kopparberg County there are in addition to Falun (13 369 and 18 453 inhabitants) a number of other towns The largest town is Borlange (17 124 and 25 009 inhabitants) and in the south-eastern part of the county there are also the towns of Ludvika (5 052 11 706) Säter (2 175 4 287) Hedemora (3 801 5 653) and Avesta (5 166 9 896) In the northern part of Älvsborg County there are three towns viz Vaners borg (8 942 inhabitants in 1930 17 511 in 1956) the nearby Trollhattan (15 014 29 317) and Åmål (6 765 8 685)

The location of the counties and hospitals selected is shown in the maps Fig 1 (p 4) and Figs 2-5 (pp 36 38 39 40)

In view of the purpose of the investigation—viz to study the clientele of DM patients at general hospitals with regard to the symptoms and course of the disease—we were anxious to secure a series that covered all existing groups by age and duration Not least were we interested in the outcome of the disease in patients belonging to the higher age groups, and especially in patients with a comparatively late onset of the disease Therefore it was clear that we ought not to restrict ourselves to studying only patients who fell ill during a limited period of time or only patients who during a limited period had reached a certain disease duration The registration covers *all patients* during a certain period—patients who applied to the hospitals in connection with the onset of DM and were diagnosed there as well as patients who came to the hospitals later on among them both persons who had migrated into the area after the onset of the disease and instances of delayed hospital registration (for instance if the patient had for several years been treated by a physician outside the hospital)

Table 1 shows the registration periods used the population of the admission areas in 1940 1950 and 1960 and the average annual number of first admissions to the hospital in question of patients afflicted with DM (inpatients as well as outpatients clinically diagnosed

Table 2 Population and number of patients by year of first admission

Hospital and area	Sex	Population thousands			Number of patients							
						Year of first admission						
		1940	1950	1960	All	1924–30	1931–35	1936–40	1941–45	1946–50	1951–55	1956–57
F Älvsborg	M	67.4	71.9	77.1	408	7	29	44	55	75	138	60
County (northern part)	F	65.3	69.5	72.3	459	3	23	33	56	107	184	53
G Kronoberg	M	77.5	81.1	81.3	304	6	11	28	40	103	116	
County	F	73.9	76.6	77.6	433	5	10	40	58	167	153	
W Kopparberg	M	125.8	135.3	145.1	470	1	13	28	49	109	200	70
County	F	122.7	131.8	141.0	566	8	15	19	63	175	200	86
Z Jämtland	M	72.1	74.3	72.0	498	2	36	68	86	113	154	39
County	F	66.7	69.8	67.8	621	7	33	60	92	171	191	67
Total	M	342.8	362.6	375.5	1 680	16	89	168	230	400	608	169
	F	328.6	347.7	358.7	2 079	23	81	152	269	620	728	206
	M+F	671.4	710.3	734.2	3 759	39	170	320	499	1 020	1 336	375

Location of areas see map Fig 1 p 4

in connection with the admission or with DM already diagnosed at another hospital or by a doctor outside the hospitals provided that the diagnosis DM was confirmed)

The scope of the clinical material

Table 2 gives the population and the number of patients by hospital sex and year of first admission to the hospital The total series covers 3 759 patients of whom 1 680 are males and 2 079 are females

In the planning of the data registration the following questions could be asked

(a) What data were available in the case records?

(b) Which data should be extracted?

(c) To what extent might there be incompleteness in the registration?

(d) Which data were of particular interest to research workers dealing with different aspects of DM?

(e) What number of cases of different types could one expect to find and what possibilities might there be of arriving at conclusive results with a series of the size to be expected? (At that time we did not know the exact number of patients, given in Table 2 but our assessment proved to be fairly correct)

(f) What comparisons in respect of the occurrence of disease in the general population might be needed and what data could be taken from other sources?

(g) How complete should the extracts from the case records be and to what extent should we take the risk of possibly being compelled to go back to the primary data for supplementary registration?

(h) In what respects should the data from the case records be supplemented

by additional information from other quarters?

(i) How should the extracts from the case records be arranged?

(j) In what way should these extracts be grouped so as to be easily handled?

(k) What definitions and divisions should be applied in the processing of the material?

In view of our general knowledge of hospital records and population statistics in Sweden our special knowledge of the selected hospitals scrutinies of the case records and the character of DM, we were aware that the final processing of the data should be made with a division by *sex* and by at least two of the three variables age at onset the patient s age and the duration of the disease Taking into acount the ages and durations to be expected we found it most suitable to use differences between calendar years—hence to define *age* as the difference between the actual year and the patient s birth year and *duration* as the difference between the actual year and the year of onset We decided to use 5 year groups and to make the tabulations with the two arguments *age at onset* and *duration of the disease*

We were aware that the time (the epoch) for a certain registration in the case records might play a considerable part—with regard to the nature and completeness of entries and in several other respects Therefore the *epoch* (the calendar year) was included in the registration

We were aware that there could exist differences between the four *hospitals* in part due to the age composition of the population in the admission areas, the communications etc

The scope of the extracts to be made was decided upon in the course of thorough discussions between the physicians-in-chief of the hospitals, several other physicians and statistical experts Dr Carl Philipson devoted much time to elaborating the plan for the code lists and the technical methods for the processing of the data He also gave instructions and surveyed the coding work We take this opportunity to express our sincere thanks to Dr Philipson for his most valuable contributions during this part of the investigation

With regard to every occasion (admission) concerning which there were relevant entries in the case records the data were extracted and converted into punched cards (*occasion cards*) The instructions for these extracts comprise the following headings (the classifications used will be evident from the following chapters)

Hospital
Sex
Date of birth
Year of onset of DM
Year of occasion
Marital state
Height
Weight
Constitutional type
Working capacity
Blood pressure systolic
Blood pressure diastolic
Visits to hospitals
Glycosuria
Hyperglycaemia
Hypoglycaemia
Acidosis
Non protein nitrogen
Creatinine in blood

Serum protein
Other laboratory tests

Infections
Tumours
Liver diseases
Rheumatoid arthritis
Allergic diseases
Vascular changes
Cushing s syndrome
Thyrotoxicosis
Other endocrine dysfunctions

Dietetic prescriptions
Insulin type
Insulin quantity per day
Insulin dosages per day

Retinopathy
Nephropathy
Neuropathy

Menarche
Menstruation frequency and intensity
Libido potency
Menopause
Partus month
Delivery complications
Weight of child

Diabetic relatives (specified)

In addition the extracts contained information on the name and address of the patient and the number of the case record

To a certain extent the case record data were supplemented by interrogating the patients still under control (this was done at Vanersborg in particular) For a certain number of patients who prior to their admission to the hospital had been treated at other hospitals or by physicians outside the hospitals supplementary information was obtained and included in the occasion cards however, annotation was always carried out with regard to the year of first admission to the hospital

On the basis of the occasion cards the data were processed to give a summary of the registrations during each 5-year period of duration reckoned from the onset of DM Hence the first duration period covers the calendar year of onset and the subsequent four calendar years the second duration period covers the following five calendar years and so on In the main in addition to the year and age of onset (occasion card 0) and the year and age of first admission we wanted to study the state (*the status*) of the patient during the duration period—either the maximum deviation from normal or the average for the occasions registered within the period—and *the change* observed within the period in comparison with the observations during the preceding one

As already mentioned the number of patients was 3 759 The total number of admissions (first admissions and re-admissions) was 45 217 and the total number of registration periods (duration cards) was 7,385 In all the series may be said to represent about 19,700 observation years or rather more than 5 years per patient (cf p 60)

The distribution of the patients by sex hospital age at first admission to the hospital and year of first admission is shown in Table 3 It should be noticed that the low numbers for first admissions during the 1950s of patients below age 15 is ascribable to the fact that the registration does not comprise the paediatric departments in Sweden there have been made a series of thorough investigations concerning juvenile hospital patients with DM and we therefore thought it advantageous to concentrate

Table 3 Population and number of patients by age at and year of first admission

Sex and age at first admission	Hospital and area	Population thousands			Number of patients							
					All	Year of first admission						
		1940	1950	1960		1924–30	1931–35	1936–40	1941–45	1946–50	1951–55	1956–57
Males												
0–14	P	13 6	16 2	17 4	35	1	10	8	8	8	–	–
	G	16 7	18 7	17 8	31	2	2	4	10	12	1	
	W	24 6	31 6	33 4	26	–	4	5	7	7	3	–
	Z	16 4	19 0	16 6	26	–	6	8	6	4	2	–
	Total	71 3	85 5	85 2	118	3	22	25	31	31	6	–
15–39	P	27 6	25 5	25 2	124	5	15	15	[illegible]	23	36	12
	G	31 1	28 0	25 5	85	4	8	13	[illegible]	24	26	
	W	51 9	46 9	47 0	148	–	5	11	22	34	64	12
	Z	30 1	26 1	22 8	102	–	12	19	26	22	17	6
	Total	140 7	126 5	120 5	459	9	40	58	76	103	143	30
40–64	P	18 7	22 5	25 6	164	1	4	18	26	33	55	27
	G	21 5	25 0	27 2	117	–	1	8	15	46	47	
	W	36 7	42 8	47 8	220	1	4	10	15	30	106	34
	Z	19 2	21 7	23 5	221	2	15	27	35	51	75	16
	Total	96 1	112 0	124 1	722	4	24	63	91	180	283	77
65–	P	6 5	7 7	8 9	85	–	–	3	3	11	47	21
	[illegible]	8 2	9 4	10 8	71	–	–	3	5	21	42	
	W	11 6	14 0	16 9	76	–	–	2	5	18	27	24
	Z	6 4	7 5	9 1	149	–	3	14	19	36	60	17
	Total	32 7	38 6	45 7	381	–	3	22	32	86	176	62
Females												
0 14	P	12 9	15 3	15 3	30	2	5	10	7	6	–	–
	G	15 8	17 6	16 9	29	3	2	7	10	6	1	
	W	23 9	30 3	31 5	29	1	5	4	8	10	1	–
	Z	15 6	18 2	15 6	27	2	4	6	11	4	–	–
	Total	68 2	81 4	79 3	115	8	16	27	36	26	2	–
15 39	P	25 9	23 7	23 3	84	1	9	6	12	18	26	12
	G	27 8	25 4	24 4	65	2	4	6	4	25	24	
	W	40 3	44 8	44 6	117	5	5	4	20	39	34	10
	Z	26 7	24 1	21 8	64	1	8	11	8	13	16	7
	Total	120 7	118 0	114 1	330	9	26	27	44	95	100	29
40–64	P	19 0	22 2	24 1	212	–	9	14	31	59	92	27
	G	21 3	23 8	25 1	183	–	4	22	36	65	56	
	W	37 3	42 2	46 5	274	2	5	8	31	91	98	39
	Z	18 1	20 5	21 6	267	3	15	26	42	79	79	23
	Total	95 7	108 7	117 3	956	5	33	70	140	294	325	89
65–	P	7 5	8 3	9 6	113	–	–	4	6	24	65	14
	G	9 0	9 8	11 2	156		–	5	8	71	72	
	W	12 2	14 5	18 4	146		–	3	4	35	67	37
	Z	6 2	7 0	8 8	263	1	6	17	31	75	96	37
	Total	34 9	39 6	48 0	678	1	6	29	49	205	300	88

Table 4 Aggregate admission rates 1946–50 and 1951–55

	Sex	Age limit	P		G		W		Z	
			1946–50	1951–55	1946–50	1951–55	1946–50	1951–[illegible]	1946–50	1951–55
First admissions (residents within the area)	M		74	130	100	116	107	195	111	154
	F		103	178	166	151	173	199	169	191
Aggregate admission rate per cent	M	40	0 7	0 6	0 6	0 5	0 5	0 4	0 6	0 3
		50	1 0	1 0	0 9	0 7	0 7	1 0	0 8	0 6
		65	1 3	1 8	1 5	1 4	1 1	1 7	1 9	2 1
		80	1 6	3 6	2 1	2 9	1 4	2 3	3 4	4 5
	F	40	0 5	0 5	0 6	0 4	0 5	0 4	0 4	0 4
		50	0 8	0 9	0 9	0 7	0 7	0 6	0 6	0 7
		65	1 8	2 7	2 0	1 8	1 6	1 8	2 5	2 5
		80	2 7	5 1	4 1	3 9	2 2	3 0	5 8	6 7

our study exclusively on patients admitted to departments of internal medicine The increase in the number of first admissions with time especially in the higher age groups is in part a reflection of the changing age composition of the population (and the increase in population number) but in the main it is ascribable to other factors, such as better communications augmented hospital facilities and a general rise in "the consumption standards in respect of medical care, this last mentioned phenomenon is *seen for many other types* of disease, and it should not be interpreted as an expression of an increase by time of the incidence of DM (for a certain group by sex and age) This *question will* be examined in a later connection

Using the admission figures for the periods 1946–50 and 1951–55 as shown in Table 3 (but with a division by 5 year age groups) and the data concerning the age distribution of the population in the admission areas (viz the northern part of Älvsborg County and the whole of the counties of Kronoberg, Kopparberg and Jamtland) *the aggregate admission rates* up to certain ages for the two periods have been calculated in Table 4, in principle, these rates show the probability that a person in the area will be admitted to the hospital and diagnosed as being afflicted with clinically manifest DM, provided (1) *that he does not* 'escape' by dying before the age mentioned and before admission takes place, and (2) that the circumstances with regard to admission remain throughout as they were during the period in question (Hence, in this latter respect, the suppositions are similar to those applied when calculating a mortality table for a certain period of time) As will be seen from the table, for the period 1951–55 the aggregate admission rate up to age 80 was 4 5 per cent for males and 6 7 per cent for females in Jamtland County and 3 6 and 5 1 per cent, respectively, in the northern part of Älvsborg County, but was considerably lower in Kronoberg County (2 9 and 3 9 per cent) and in Kopparberg County (2 3 and 3 0 per cent) However

Table 5 Age at onset, first registration and first admission to investigation hospital

Sex and age	Hospital P			G			W			Z			Total		
	O	R	A	O	R	A	O	R	A	O	R	A	O	R	A
Males	408	408	408	304	304	304	470	470	470	498	498	498	1 680	1 680	1 680
0– 4	8	7	7	11	4	4	6	–	–	4	2	2	[illegible]	13	13
5– 9	19	20	16	12	10	10	[illegible]	6	6	9	9	9	59	45	41
10–14	[illegible]	20	12	[illegible]	17	17	26	20	20	23	11	15	95	75	64
15–19	25	25	27	20	23	21	34	28	28	15	15	17	94	91	93
20–24	14	15	17	13	20	21	27	28	28	24	24	23	78	87	89
25–29	24	24	28	15	12	12	28	33	33	22	21	20	89	90	93
30–34	27	27	29	15	17	17	34	28	27	20	17	19	96	89	92
35–39	27	26	23	14	14	14	30	31	32	22	[illegible]	23	93	[illegible]	92
40–44	32	31	36	19	20	19	41	39	37	26	24	24	118	114	116
45–49	28	25	[illegible]	24	22	21	49	43	45	30	30	28	131	120	116
50–54	36	37	31	28	22	25	49	53	53	41	39	39	154	151	148
55–59	39	37	42	27	29	28	45	40	40	63	60	56	174	166	166
60–64	31	34	33	19	23	24	33	51	45	63	68	74	146	176	176
65–69	38	38	43	23	24	22	25	32	38	50	55	50	136	149	153
70–74	24	26	24	20	24	23	20	30	30	40	40	43	104	120	120
75–79	13	15	17	15	16	18	3	6	6	34	40	40	65	77	81
80–84	–	–	–	5	6	7	1	2	2	12	14	15	18	22	24
85–89	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
90–	–	1	1	1	1	1	–	–	–	–	–	–	1	2	2
Females	459	459	459	433	433	433	566	566	566	621	621	621	2,079	2,079	2,079
0– 4	7	5	5	5	4	4	8	4	4	3	2	2	23	15	15
5– 9	19	17	13	16	13	12	24	11	9	14	13	13	73	54	47
10–14	16	17	12	19	12	13	23	15	16	15	12	12	73	56	53
15–19	17	16	18	14	9	9	21	25	22	15	15	15	67	65	64
20–24	17	15	15	18	18	18	24	20	21	12	9	9	71	62	63
25–29	14	11	15	10	15	15	20	25	26	11	14	13	55	65	69
30–34	19	21	24	9	10	9	27	25	27	9	11	12	64	67	72
35–39	12	12	12	15	14	14	19	21	21	16	16	15	62	[illegible]	62
40–44	17	14	14	19	13	13	37	25	25	20	20	19	93	72	71
45–49	36	40	40	36	33	33	40	41	40	29	24	23	141	138	136
50–54	52	52	52	44	37	36	67	52	52	58	47	48	221	188	188
55–59	66	66	64	48	46	45	85	76	77	83	83	82	282	271	268
60–64	60	62	62	48	58	56	[illegible]	[illegible]	80	102	103	95	273	304	293
65–69	46	47	49	[illegible]	66	69	[illegible]	68	69	107	114	112	279	295	299
70–74	36	37	35	40	51	50	32	59	58	79	82	[illegible]	187	229	228
75–79	21	22	[illegible]	24	26	29	11	15	15	39	45	52	95	108	119
80–84	3	4	4	4	6	6	1	2	3	5	7	9	13	[illegible]	22
85–89	1	1	2	1	2	2	1	1	1	4	4	5	7	8	10

O = Onset R = First registration A = First admission to investigation hospital

as will be shown below, there are considerable differences in respect of the extent to which the hospitals cover different parts of their admission areas

Table 5 gives the distribution by sex and hospital of age at onset, age at first registration and age at first admission to the investigation hospital (with 5-year

Table 6 . Average interval from onset to first registration and first admission

Average interval from onset years	Hospital								Total		
	P		G		W		Z				
	M	F	M	F	M	F	M	F	M	F	M+F
First registration (R – O)	0 7	0 9	2 0	2 6	4 0	3 5	1 5	1 0	2 1	1 9	2 0
First admission (A – O)	1 5	1 3	2 3	2 8	4 1	3 6	1 8	1 4	2 5	2 2	2 3

age groups) and in Table 6 are shown the average age differences between first registration and onset, and between first admission and onset The average time that elapsed from onset to first registration is 2 0 years and the average time from onset to first admission is 2 3 years The intervals are shortest at Vanersborg and are short at Östersund also, whereas they are considerably longer at Falun

Tables 7–10 give an account of the composition of the registrations (the duration cards)

In Table 7B for each of the four hospitals the number of registrations by sex and 5 year periods is shown for 5 year classes by age at onset and for the three types by age at onset mentioned already, viz 0–14 (juvenile onset) 15–39 (early adult onset) and 40 or over (late onset) In addition the table gives an account of the number of patients by age at onset—for all patients and for those who have not been registered within the first duration group (0–4 years)

In Table 7A the data of Table 7B are concentrated and rearranged showing by sex and the three types by age at onset the number of registrations within different duration groups It should be noticed that in the highest duration group (25 years and over) a patient may occur more than once (for instance if there are registrations both with duration 25–29 and with duration 30–34)

For the three types by age at onset (juvenile, early adult late) Table 8 gives the mean age by sex, hospital and 5-year duration groups (0–24 years) Because of the rise with time in the number of first admissions, and the correlation between mortality and age, there is among patients with late onset DM only a comparatively small increase of the mean age with duration Further, it should be noted that among these patients the mean age is a good deal higher at Östersund (Z) and lower at Falun (W) than corresponds to the average for the four hospitals taken together

In Table 9 the data of Table 7A are split into two parts being grouped according to whether the first calendar year within the registration period falls before 1950 or after 1949 As will appear in a later connection, the findings concerning the occurrence of late complications show conspicuous divergences between the hospitals and still more conspicuous divergences between the observations from different periods of time (epochs) In view of the scope of the material we have chosen to divide the series by epoch into two parts of suitable size—3 288 registrations (duration cards)

Table 7A : Registrations by age groups at onset and duration of DM

Age at onset	Hospital	Males (registrations)							Females (registrations)						
		All	Duration						All	Duration					
			0–4	5–9	10–14	15–19	20–24	25		0–4	5–9	10–14	15–19	20–24	25
0–14	P	197	47	43	40	35	18	14	150	39	37	32	21	10	11
	G	120	33	32	26	17	6	6	112	28	29	29	14	8	4
	W	129	23	29	30	23	17	7	136	31	26	32	20	17	10
	[illegible]	102	30	22	25	12	7	6	87	27	23	21	13	2	1
	Total	548	133	126	121	87	48	33	485	125	115	114	68	37	26
15–39	P	350	115	86	59	39	24	27	208	73	54	35	17	11	[illegible]
	G	197	66	47	30	28	18	8	148	42	33	28	20	13	12
	W	315	115	70	51	34	17	28	271	82	65	45	32	26	21
	Z	213	90	49	33	23	12	6	145	54	34	23	19	8	7
	Total	1 075	386	252	173	124	71	69	772	251	186	131	88	58	58
40–	P	480	234	129	64	35	12	6	644	336	191	76	26	9	[illegible]
	G	300	165	79	32	14	8	2	588	286	175	83	36	7	1
	W	425	214	113	58	29	8	3	667	312	201	96	46	11	1
	Z	570	331	148	58	23	9	1	831	489	215	85	34	[illegible]	2
	Total	1 775	944	469	212	101	37	12	2 730	1 423	782	340	142	33	10
Total	P	1 027	396	258	163	109	54	47	1 002	448	282	143	64	30	35
	G	617	264	158	88	59	32	16	848	356	237	140	70	[illegible]	17
	W	869	352	212	139	86	42	38	1 074	425	292	173	[illegible]	54	32
	Z	885	451	219	116	58	28	13	1 063	570	272	129	66	16	10
	Total	3 398	1 463	847	506	312	156	114	3 987	1 799	1 093	585	298	128	94

in the earlier epoch and 4 097 registrations in the later epoch

To a great extent the analysis of the clinical findings will be made in the form of standard comparisons the calculation of the expectations being based on total frequencies (by sex age at onset, and duration) and the numbers (by hospital etc) given in Tables 7–9

In order to be able to analyse the mortality in our hospital series all patients who were not registered as deceased in the case records and had not visited the hospitals after the end of the observation period were traced with the aid of the population registers and if necessary, by means of letters to the incumbents of their home parishes, until it could be ascertained whether they were living at the end of the investigation period or had died (in which case information on the date of death was obtained)

Table 10 gives a survey of the course of registration of the patients (by sex and the three types by age at onset) As will be seen from the table 3 262 patients were registered with duration 0–4 years Of these 269 died within the duration period (before January 1 1956) Another 47 patients moved during the period (ending before 1956) they were not registered in a later period but it was ascertained that they were living on January 1, 1956 A further 1,111 patients belonged to a period ending after 1955 and were living on January 1 1956 The

Table 7B Patients by age at onset, and registrations by age at onset and duration of DM

Hospital and age at onset		Patients M All	Patients M Not dur 0–4	Patients F All	Patients F Not dur 0–4	Registrations (in 5 year period) duration M 0–4	M 5–9	M 10–14	M 15–19	M 20–24	M 25–	F 0–4	F 5–9	F 10–14	F 15–19	F 20–24	F 25–
P	0–4	8	–	7	2	8	8	8	8	1	–	5	5	5	4	4	4
	5–9	19	–	19	1	19	[illegible]	19	14	7	2	18	16	16	12	3	5
	10–14	23	3	16	–	20	17	13	13	10	12	16	16	11	5	3	[illegible]
	15–19	25	–	17	1	25	[illegible]	12	8	6	8	16	10	8	3	3	4
	20–24	14	1	17	2	13	11	7	5	3	4	15	14	9	4	2	4
	25–29	24	–	14	1	24	21	13	11	8	5	13	8	4	2	3	8
	30–34	27	–	19	1	27	23	16	6	5	7	18	14	9	5	[illegible]	1
	35–39	27	1	12	1	26	13	11	9	2	3	11	8	5	3	1	1
	40–44	32	2	17	1	30	23	20	10	4	–	16	12	8	4	1	1
	45–49	28	2	36	–	26	17	8	5	2	1	36	22	9	6	4	3
	50–54	36	–	52	–	36	21	14	9	3	2	52	37	17	4	2	2
	55–59	39	2	66	1	37	28	12	7	3	3	65	37	24	8	2	–
	60–64	31	1	60	–	30	15	5	3	–	–	60	34	8	–	–	–
	65–69	38	–	46	–	38	10	4	1	–	–	46	21	5	1	–	
	70–74	24	–	36	–	24	12	–	–	–	–	36	20	3	2	–	–
	75–79	13	–	31	–	13	3	1	–	–	–	21	8	2	1	–	–
	80–84	–	–	3	–	–	–	–	–	–	–	3	–	–	–	–	–
	85–89	–	–	1	–	–	–	–	–	–	–	1	–	–	–	–	–
	0–14	50	3	42	3	47	43	40	35	18	14	39	37	32	21	10	11
	15–39	117	2	79	6	115	86	59	39	24	27	73	54	35	17	11	18
	40–	241	7	338	2	234	129	64	35	12	6	336	191	76	26	9	6
	Total	408	12	459	11	396	258	163	109	54	47	448	282	143	[illegible]	30	35
G	0–4	11	6	5	1	5	4	6	4	2	[illegible]	4	4	4	3	1	1
	5–9	12	3	16	2	9	9	10	7	2	–	14	11	13	8	5	–
	10–14	23	4	19	9	19	19	10	6	2	–	10	14	12	3	2	3
	15–19	20	2	14	7	[illegible]	16	10	12	8	2	7	[illegible]	6	3	3	5
	20–24	13	1	18	5	12	8	5	4	3	2	13	9	6	7	4	2
	25–29	15	3	10	5	12	8	6	6	5	4	5	4	4	2	2	5
	30–34	15	2	9	1	13	6	5	3	1	–	8	6	4	4	1	–
	35–39	14	3	15	6	11	9	4	3	1	–	9	6	8	4	3	–
	40–44	19	2	19	4	17	12	9	6	2	–	15	14	7	3	1	1
	45–49	24	3	36	9	21	15	6	3	3	–	27	24	18	9	1	–
	50–54	28	7	44	9	21	12	7	[illegible]	2	2	35	35	23	10	4	–
	55–59	27	2	48	9	25	12	6	3	1	–	39	28	17	8	1	–
	60–64	19	–	48	3	19	7	2	–	–	–	45	28	10	4	–	–
	65–69	23	2	63	6	21	13	2	–	–	–	57	24	6	1	–	–
	70–74	20	–	40	–	20	6	–	–	–	–	40	14	2	1	–	–
	75–79	15	–	24	1	15	2	–	–	–	–	23	8	–	–	–	–
	80–84	5	–	4	–	5	–	–	–	–	–	4	–	–	–	–	–
	85–89	–	–	1	–		–	–	–	–	–	1	–	–	–	–	–
	90–	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
	0–14	46	13	40	12	33	32	26	17	6	6	28	29	29	14	8	4
	15–39	77	11	66	24	66	47	30	28	18	8	42	33	28	20	13	12
	40–	181	16	327	41	165	79	32	14	8	2	286	175	83	36	7	1
	Total	304	40	433	77	264	158	88	59	32	16	356	237	140	70	28	17

Table 7B Continued

Hospital	Age at onset	Patients M All	Patients M Not dur 0–4	Patients F All	Patients F Not dur 0–4	Registrations (in 5-year period) duration M 0–4	M 5–9	M 10–14	M 15–19	M 20–24	M 25–	F 0–4	F 5–9	F 10–14	F 15–19	F 20–24	F 25–
W	0–4	[illegible]	6	8	4	–	2	2	2	4	1	4	4	4	4	4	3
	5–9	19	12	24	12	7	10	12	12	[illegible]	5	12	11	[illegible]	8	[illegible]	2
	10–14	26	10	23	8	16	17	16	9	5	1	15	11	10	8	7	5
	15–19	34	13	21	5	21	9	11	11	7	17	16	10	4	5	6	7
	20–24	27	4	24	10	23	14	12	7	4	2	14	14	12	10	7	5
	25–29	28	6	20	3	22	15	8	4	2	5	17	13	9	2	2	2
	30–34	34	9	27	7	25	16	9	4	–	2	20	18	11	10	6	5
	35–39	30	6	19	4	24	16	11	8	4	2	15	10	9	5	5	2
	40–44	41	10	27	16	31	22	15	6	2	3	21	22	15	12	5	1
	45–49	49	11	40	11	38	20	11	9	3	–	29	23	15	7	4	–
	50–54	49	11	67	[illegible]	38	21	15	9	3	–	49	36	23	14	1	–
	55–59	45	10	85	17	35	17	9	4	–	–	68	48	22	11	1	–
	60–64	33	2	63	13	31	15	4	1	–	–	50	33	14	2	–	–
	65–69	25	5	63	11	20	13	3	–	–	–	52	29	[illegible]	–	–	–
	70–74	20	3	32	2	17	5	1	–	–	–	30	8	1	–	–	–
	75–79	3	–	11	–	3	–	–	–	–	–	11	2	–	–	–	–
	80–84	1	–	1	–	1	–	–	–	–	–	1	–	–	–	–	–
	85–89	–	–	1	–	–	–	–	–	–	–	1	–	–	–	–	–
	0–14	51	28	55	24	23	29	30	23	17	7	31	26	32	20	17	10
	15–39	153	38	111	29	115	70	51	34	17	28	82	65	45	32	26	21
	40–	266	52	400	[illegible]	214	113	58	29	8	3	312	201	96	46	11	1
	Total	470	118	566	141	352	212	139	86	42	38	425	292	173	98	54	32
Z	0–4	4	1	3	1	3	2	3	3	1	–	2	1	3	1	–	–
	5–9	9	–	14	2	9	6	7	2	2	1	12	10	9	7	–	1
	10–14	23	5	15	2	18	14	15	7	4	5	13	12	[illegible]	5	2	–
	15–19	15	1	15	1	14	5	3	4	1	–	14	[illegible]	[illegible]	5	1	–
	20–24	24	4	12	4	20	9	10	7	4	3	[illegible]	8	3	3	3	4
	25–29	22	1	11	3	21	10	8	4	1	–	8	8	4	4	2	2
	30–34	20	5	9	–	15	11	7	5	3	1	9	2	2	2	1	1
	35–39	22	2	16	1	20	14	5	3	3	2	15	7	8	5	1	–
	40–44	26	5	20	1	21	10	5	5	3	1	19	13	6	4	1	2
	45–49	30	4	29	5	26	18	8	3	2	–	24	19	9	6	1	–
	50–54	41	5	58	11	36	19	11	4	1	–	47	35	22	11	3	–
	55–59	63	6	83	6	57	28	16	7	1	–	77	41	23	6	1	–
	60–64	63	1	102	11	62	25	9	3	2	–	91	43	14	3	–	–
	65–69	50	4	107	1	46	21	7	1	–	–	106	38	8	3	–	–
	70–74	40	3	79	2	37	15	1	–	–	–	77	23	3	1	–	–
	75–79	34	–	39	–	34	10	1	–	–	–	39	3	–	–	–	–
	80–84	12	–	5	–	12	2	–	–	–		5	–	–	–	–	–
	85–89	–	–	4	–	–	–	–	–	–	–	4	–	–	–	–	–
	0–14	36	[illegible]	32	5	30	22	25	12	7	[illegible]	27	23	21	13	[illegible]	1
	15–39	103	13	63	9	90	49	33	23	12	[illegible]	54	34	23	19	8	7
	40–	359	28	526	37	331	148	58	23	9	1	489	215	85	34	6	2
	Total	498	47	621	51	451	219	116	58	28	13	570	272	129	66	16	10

Table 8 : Mean age in groups by sex hospital age at onset and duration

Duration	Hospital	Juvenile onset 0 14		Early adult onset 15 39		Late onset 40	
		M	F	M	F	M	F
0– 4	P	10 3	10 4	29 7	28 5	60 1	61 9
	G	11 1	10 1	28 0	28 9	62 0	63 6
	W	12 5	10 8	29 3	29 2	57 4	61 1
	Z	11 5	11 0	29 4	29 3	63 7	65 0
	Total	11 1	10 6	29 2	29 0	61 1	63 1
5– 9	P	15 0	15 5	34 1	33 6	62 4	65 3
	G	16 3	15 7	32 3	32 9	62 8	64 7
	W	16 6	15 3	35 1	34 3	60 9	63 4
	Z	16 7	16 4	36 0	32 5	66 6	66 0
	Total	16 0	15 7	34 8	33 5	63 4	64 9
10–14	P	19 6	19 7	39 6	38 1	63 4	67 4
	G	19 8	20 4	37 0	39 4	62 8	65 9
	W	21 3	19 9	38 7	40 0	63 1	65 4
	Z	21 4	20 4	39 2	39 7	68 0	67 9
	Total	20 4	20 1	38 8	39 3	64 4	66 6
15–19	P	24 7	24 2	44 4	44 3	67 7	70 9
	G	24 6	24 0	40 6	43 8	64 7	70 1
	W	25 5	25 0	42 7	44 0	66 4	67 3
	Z	25 7	25 5	43 1	43 7	69 7	70 6
	Total	25 0	24 7	42 8	43 9	67 4	69 5
20–24	P	31 5	28 5	47 8	47 2	71 1	71 8
	G	29 0	29 6	44 6	47 8	70 2	72 6
	W	29 3	29 9	46 1	48 4	69 6	68 1
	Z	31 1	34 0	50 2	47 8	72 3	72 3
	Total	30 4	29 7	47 0	48 0	70 9	70 8

The number of registrations (patients in the duration group) is given in Table 7A

remaining 1 835 patients in duration group 0–4 years were registered in a later period too Of these 1 835 patients however only 1 695 were registered in the subsequent period (duration 5–9 years) the remaining 140 did not return until a later period In addition to the 1 695 patients from the first duration period there appeared in the second duration period 235 new patients—thus patients who paid their first visit to the hospital (with the diagnosis DM) after the disease had been manifest for 5–9 years (the difference between the calendar year of admission and the calendar year of onset) At the bottom of the table there are totals for all duration periods taken together Of the 3 759 patients, 634 died before January 1, 1956, and 101 moved but were living on that date 3,024 patients were registered in a period ending after 1955 and were still living on January 1 1956

Admission rates and domicile

Although the registration hospitals are well-equipped specialized hospitals and in many respects dominate their

Table 9 Registrations by age groups at onset and duration grouped by first calendar year within the registration period (epoch)

Age at onset	Epoch	Hospital	Males (registrations) All	Duration 0–4	5–9	10–14	15–19	20–24	25-	Females (registrations) All	Duration 0–4	5– 9	10– 14	15– 19	20– 24	25–
0–14	E1 (–1949)	P	116	43	31	20	15	3	4	92	37	29	16	7	2	1
		G	71	[illegible]	21	13	7	2	–	73	27	22	15	6	2	1
		W	68	19	21	17	8	2	1	76	30	15	12	10	7	2
		Z	[illegible]	27	17	14	7	2	1	61	27	20	10	4	–	–
		Total	323	117	90	64	37	9	6	302	121	[illegible]	53	27	11	4
	E2 (1950–)	P	[illegible]	4	12	20	20	15	10	58	2	8	16	14	8	10
		G	49	5	11	13	10	4	6	39	1	7	14	8	6	3
		W	61	4	8	13	15	15	6	60	1	11	20	10	10	8
		Z	34	3	5	11	5	5	5	26	–	3	11	9	2	1
		Total	225	16	36	57	50	39	27	183	4	29	61	41	26	22
15–39	E1 (–1949)	P	178	78	47	28	13	9	3	93	44	25	9	6	4	5
		G	112	47	28	19	15	2	1	76	27	16	17	[illegible]	4	2
		W	122	51	26	20	9	2	14	127	47	30	21	17	[illegible]	3
		Z	123	69	26	17	5	3	3	80	30	23	15	8	4	–
		Total	535	245	127	84	42	16	21	376	148	94	62	41	21	10
	E2 (1950–)	P	172	37	39	31	26	15	24	115	29	29	26	11	7	13
		G	85	19	19	11	13	16	7	72	15	17	11	10	9	10
		W	193	64	44	31	25	15	14	144	35	35	24	15	17	18
		Z	90	21	23	16	18	9	3	65	24	11	8	11	4	7
		Total	540	141	125	89	82	55	48	396	103	92	69	47	37	48
40–	E1 (–1949)	P	162	98	35	21	3	2	3	195	128	42	17	5	1	2
		G	124	79	24	12	6	3	–	282	170	66	40	6	–	–
		W	123	69	29	18	5	1	1	197	116	45	25	10	1	–
		Z	276	173	59	28	12	4	–	393	249	87	42	13	2	–
		Total	685	419	147	79	26	[illegible]	4	1 067	663	240	124	34	4	2
	E2 (1950–)	P	318	136	94	43	32	10	3	449	208	149	59	21	8	4
		G	176	[illegible]	55	20	8	5	2	306	116	109	43	30	7	1
		W	302	145	[illegible]	40	24	7	2	470	196	156	71	36	10	1
		Z	294	158	89	30	11	5	1	438	240	128	43	21	4	2
		Total	1 090	525	322	133	75	27	8	1 663	760	542	216	108	29	8
Total	E1 (–1949)	P	456	219	113	69	31	14	10	380	209	96	42	18	7	8
		G	307	154	73	44	28	7	1	431	224	104	72	22	6	3
		W	313	139	76	55	22	5	16	400	193	90	58	37	17	5
		Z	467	269	102	59	24	9	4	534	306	130	67	25	[illegible]	–
		Total	1 543	781	364	227	105	35	31	1 745	932	420	239	102	36	16
	E2 (1950–)	P	571	177	145	94	78	40	37	622	239	186	101	46	23	27
		G	310	110	85	44	31	25	15	417	132	133	[illegible]	48	22	14
		W	556	213	136	84	64	37	22	674	232	202	115	61	37	27
		Z	418	182	117	57	34	[illegible]	9	529	264	142	62	41	10	10
		Total	1 855	682	483	279	207	121	83	2 242	867	663	346	196	92	78

admission areas it is quite natural with a disease of the type that DM represents that there should occur certain geographical differences with regard to the admission rates—connected with communications (and improved communica-

Table 10 Survey of course of registration

Duration	Type of change (+ increase − decrease)	Registrations								M + F
		M				F				
		All	Age at onset			All	Age at onset			All
			0–14	15–39	40		0–14	15–39	40	
0– 4	+ New	1 463	133	386	944	1 799	125	251	1 423	3 262
	Total	1 463	133	386	944	1 799	125	251	1 423	3 262
	− Deceased	103	2	7	94	166	–	5	161	269
	− Moved	28	3	8	17	19	–	3	16	47
	− Living	504	9	104	391	607	7	70	530	1 111
	− Continued	828	119	267	442	1 007	118	173	716	1 835
5– 9	+ Continued	758	111	236	411	937	102	157	678	1 695
	+ New	89	15	16	58	146	13	29	104	235
	Total	847	126	252	469	1 083	115	186	782	1 930
	− Deceased	57	–	–	57	90	1	–	89	147
	− Moved	10	1	7	2	15	4	4	7	25
	− Living	366	21	104	241	501	20	67	414	867
	− Continued	414	104	141	169	477	90	115	272	891
10–14	+ Continued	450	106	158	186	518	100	119	299	968
	+ New	56	15	15	26	67	14	12	41	123
	Total	506	121	173	212	585	114	131	340	1 091
	− Deceased	33	1	5	27	63	8	4	51	96
	− Moved	4	1	1	2	10	5	1	4	14
	Living	207	43	64	100	263	41	52	170	470
	Continued	262	76	103	[illegible]	249	60	74	115	511
15 19	Continued	279	79	111	89	265	63	79	123	544
	New	33	8	13	12	33	5	9	19	66
	Total	312	87	124	101	298	68	88	142	610
	− Deceased	28	6	5	17	45	8	2	35	73
	− Moved	9	3	1	5	5	1	1	3	14
	− Living	147	37	56	54	139	31	33	75	286
	− Continued	128	41	62	25	109	28	52	29	237
20–24	+ Continued	137	42	64	31	113	30	53	30	250
	+ New	19	6	7	6	15	7	5	3	34
	Total	156	48	71	37	128	37	58	33	284
	− Deceased	14	3	3	8	11	4	3	4	25
	− Moved	–	–	–	–	–	–	–	–	–
	− Living	92	29	41	22	69	17	27	25	161
	− Continued	50	16	27	7	48	16	[illegible]	4	[illegible]
25–	− Continued	94	27	56	11	75	21	45	9	169
	+ New	20	6	13	1	19	5	13	1	39
	Total	114	33	69	12	94	26	58	10	208
	− Deceased	6	–	2	4	18	4	12	2	24
	− Moved	1	–	1	–	–	–	–	–	1
	− Living	71	24	41	6	58	18	35	5	129
	− Continued	36	9	25	2	[illegible]	4	11	3	54
Total	+ Continued	1 718	365	625	728	1 908	316	453	1 139	3 626
	+ New	1 680	183	450	1 047	2 079	169	319	1 591	3 759
	Total	3 398	548	1 075	1 775	3 987	485	772	2 730	7 385
	− Deceased	241	12	22	207	393	25	26	342	634
	− Moved	52	[illegible]	[illegible]	26	49	10	[illegible]	30	101
	− Living	1 387	163	410	814	1 637	134	284	1 219	3 024
	− Continued	1 718	365	625	728	1 908	316	453	1 139	3 626

tions, for instance the development of motoring) the capacity and location of other hospitals etc , and connected with the age and occupation of the patient the occurrence of intercurrent disease, and so on

For the admission areas of the four hospitals the geographical distribution of the domiciles of the patients is shown in Table 11 The divisions used are (the present) towns and certain rural districts (*härad tingslag*, mainly based on the socio-economic geographical grouping applied at the census of 1930) By sex, the table shows the population in 1950, and the number of first admissions (all, and during the periods 1946–50 and 1951–55) In the columns to the right are given certain relative figures viz the ratio all first admissions population in 1950

On the basis of the location of the towns and districts, and the quotients mentioned, the towns and districts have been taken together as indicated in Table 11 on the left (cf Figs 2–5) For these areas (four in Kopparberg County three in each of Älvsborg Kronoberg and Jamtland Counties) Table 12 gives the population by sex and age in 1950 and the number of first admissions during the period 1946–55 of residents within the area by sex and age On the basis of these data the aggregate admission rates up to certain ages have been calculated they are shown at the bottom of Table 12

For area P1 (the towns of Vänersborg and Trollhättan) the aggregate admission rates up to age 80 were 4 0 per cent for males and 6 5 per cent for females For area P2 (the rural districts of the province of Västergotland which are covered by Vänersborg Hospital) the figures are lower viz 3 0 and 3 7 per cent and for area P3 (the province of Dalsland) they are still lower or 2 0 and 2 6 per cent

Växjo (area G1) is a comparatively small town, but the figures agree well with those found for Vänersborg and Trollhättan (P1) Area G3 (the south western part of Kronoberg County, with the town of Ljungby) shows very low admission rates (0 7 and 1 2 per cent) this can be explained by the fact— known before the investigation started—that DM patients from this area are regularly admitted to the hospital at Ljungby For the rest of Kronoberg County the aggregate admission rates up to age 80 were 3 1 per cent for males and 4 7 per cent for females

For Falun town (W1) the corresponding rates are 5 1 and 6 1 per cent For the south-eastern part of the county (Vasterbergslagen W4) the figures are very low 0 9 and 0 7 per cent in this area the great majority of the DM patients go to the hospital at Ludvika

Table 10 Explanations

+ New	=	Not registered in earlier period
+ Continued	=	Registered in earlier period
– Deceased	=	Not registered in later period deceased before January 1 1956
– Moved	=	Period ending before January 1 1956 not registered in later period living on January 1 1956
– Living	=	Period ending after December 31 1955 living on January 1 1956
– Continued	=	Registered in later period

Table 11 Population and first admissions geographical distribution

District and location		Name of town or rural area	Population 1950 thousands		First admissions 1946 50		1951 55		All		First admissions (all) per thousand population (1950)	
			M	F	M	F	M	F	M	F	M	F
P1	a	Vanersborg town	7 8	8 5	4	17	25	23	59	71	7	9
	b	Trollhattan town	12 2	11 9	14	32	28	50	88	121	7	10
P2	c	Flundre Vane Bjärke	11 3	10 5	13	11	24	37	78	83	7	8
	d	Ale	8 3	7 8	10	8	13	15	34	30	4	4
P3	e	Sundal Valbo Nordal	14 8	13 9	15	17	21	23	68	61	5	4
	f	Åmål town	4 0	4 1	7	8	7	10	20	26	5	6
	g	Tossbo Vedbo	13 5	12 8	11	10	12	20	48	51	4	4
		Outside P			1	4	8	6	13	16		
		Total	71 9	69 5	75	107	138	184	408	459	6	7
G1	a	Vaxjo town	9 3	10 8	19	32	17	29	48	76	5	7
G2	b	Kinnevald	7 1	6 6	11	25	9	14	27	54	4	7
	c	Konga	12 6	11 5	21	20	18	23	61	67	5	6
	d	Uppvidinge	12 8	11 6	13	25	16	28	48	83	4	7
	e	Norrvidinge	4 1	3 8	6	12	11	12	18	30	4	8
	f	Allbo	15 5	14 3	25	42	36	32	82	91	5	7
G3	g	Sunnerbo (incl Ljungby town)	19 7	18 0	5	10	9	13	17	29	1	2
		Outside G			3	1	–	2	3	3		
		Total	81 1	76 6	103	167	116	153	304	433	4	6
W1	a	Falun town	7 8	9 1	16	25	26	30	68	85	9	9
W2	b	Falu norra tingslag	10 9	10 6	12	19	14	21	41	59	4	6
	c	Nedansiljan	18 5	18 5	17	29	28	40	75	103	4	6
	d	Ovansiljan	18 7	17 2	5	6	19	12	29	24	2	1
W3	e	Nås Malung	14 8	13 9	4	12	9	12	27	39	2	3
	f	Borlange town	10 9	10 8	17	37	25	20	70	84	6	7
	g	Falu sodra tingslag	7 2	6 7	10	10	12	17	30	47	4	7
	h	Avesta Sater Hedemora towns	8 4	8 9	12	15	19	17	46	51	5	6
	i	Folkare Hedemora tingslag	16 9	16 2	9	12	25	21	46	46	3	3
W4	j	Ludvika town	5 4	4 9	4	4	8	4	14	10	3	2
	k	Vasterbergslagen	15 8	15 0	1	4	10	5	15	14	1	1
		Outside W			2	2	5	1	9	4		
		Total	135 3	131 8	109	175	200	200	470	566	3	4
Z1	a	Östersund town	10 2	11 4	22	39	29	34	93	122	[illegible]	11
Z2	b	Revsund Brunflo Nas	9 5	8 7	12	20	13	39	56	94	6	11
	c	Ragunda	8 1	7 4	13	22	14	16	52	62	6	9
	d	Hammerdal	8 8	7 9	11	13	20	25	48	64	5	8
	e	Lit Rodon	9 5	8 8	15	25	19	19	64	88	7	10
	f	Jamtlands vastra tingslag	15 3	14 6	26	31	42	45	131	133	8	9
	g	Berg	4 2	3 6	8	14	10	12	30	42	7	11
Z3	h	Sveg, Hede	8 7	7 4	4	5	7	1	16	13	[illegible]	2
		Outside Z			2	2	–	–	8	3		
		Total	74 3	69 8	113	171	154	191	498	621	7	9

Location of areas see maps Figs 2-5 pp 36 38 39 40

Table 12 Population 1950, first admissions 1946–55 and aggregate admission rates 1946–55 by district

Sex	Age	District P1	P2	P3	G1	G2	G3	W1	W2	W3	W4	Z1	Z2	Z3
Population 1950 thousands														
M	0–14	4.7	4.5	7.0	2.3	11.8	4.6	1.9	11.2	13.7	4.8	2.7	14.2	2.1
	15–39	7.9	6.7	10.9	3.6	17.7	6.7	2.9	16.1	20.2	7.7	4.0	19.0	3.1
	40–49	2.9	3.0	4.8	1.3	7.6	2.8	1.2	7.0	8.7	3.2	1.3	7.4	1.2
	50–64	2.9	3.2	5.7	1.4	8.7	3.2	1.2	8.2	9.8	3.5	1.5	8.9	1.4
	65–	1.6	2.2	3.9	0.7	6.3	2.4	0.6	5.6	5.8	2.0	0.7	5.9	0.9
	Total	20.0	19.6	32.3	9.3	52.1	19.7	7.8	48.1	58.2	21.2	10.2	55.4	8.7
F	0–14	4.5	4.1	6.7	2.2	11.2	4.2	1.8	10.8	13.2	4.5	2.5	13.7	2.0
	15–39	7.9	6.1	9.7	4.4	15.2	5.8	3.5	15.1	19.2	7.0	4.6	16.9	2.6
	40–49	3.0	2.7	4.4	1.5	6.8	2.5	1.3	6.7	8.2	2.9	1.6	6.7	1.0
	50–64	3.1	3.2	5.8	1.6	8.3	3.1	1.6	8.2	9.8	3.5	1.8	8.3	1.1
	65–	1.9	2.2	4.2	1.1	6.3	2.4	0.9	5.5	6.1	2.0	0.9	5.4	0.7
	Total	20.4	18.3	30.8	10.8	47.8	18.0	9.1	46.3	56.5	19.9	11.4	51.0	7.4
First admissions 1946–55 (residents within the area)														
M	0–14	–	1	7	2	9	2	1	2	4	3	1	5	–
	15–39	17	16	24	11	32	6	11	29	46	8	4	29	6
	40–49	10	10	6	3	22	2	10	11	31	6	2	23	2
	50–64	21	15	20	12	49	3	13	32	45	5	27	69	1
	65–	23	18	16	8	54	1	7	21	16	1	17	77	2
	Total	71	60	73	36	166	14	42	95	142	23	51	203	11
F	0–14	3	2	1	1	5	1	2	2	7	–	–	4	–
	15–39	18	11	12	13	30	4	5	25	37	6	10	18	–
	40–49	9	12	12	4	23	2	7	8	21	3	6	17	1
	50–64	57	22	34	15	65	11	27	46	68	7	29	104	1
	65–	35	24	29	28	110	5	14	46	40	1	[illegible]	138	4
	Total	122	71	[illegible]	61	233	23	55	127	173	17	73	281	6
Aggregate admission rate 1946–55 per cent														
M	40	0.6	0.7	0.7	0.9	0.6	0.3	1.1	0.5	0.7	0.4	0.3	0.4	0.4
	50	1.0	1.1	0.9	1.2	0.8	0.4	1.9	0.7	1.0	0.6	0.5	0.7	0.6
	65	2.1	1.8	1.4	2.5	1.7	0.6	3.5	1.3	1.7	0.8	3.3	2.0	0.7
	80	4.0	3.0	2.0	4.2	3.1	0.7	5.1	1.8	2.3	0.9	7.0	3.9	1.0
F	40	0.7	0.6	0.4	0.8	0.6	0.3	0.6	0.5	0.6	0.2	0.5	0.4	0.0
	50	1.1	1.0	0.6	1.2	0.9	0.4	1.1	0.6	0.9	0.3	1.0	0.6	0.1
	65	3.8	2.1	1.5	2.5	2.1	0.9	3.6	1.5	2.0	0.6	3.5	2.6	0.3
	80	6.5	3.7	2.6	6.2	4.7	1.2	6.1	2.7	3.0	0.7	7.8	6.4	1.0

For the remainder of the county there are considerable local differences as can be seen from Table 11, on the whole the figures are similar to those found for Dalsland but are lower than in the Vastergotland part of Älvsborg County and in the main admission area for Växjo Hospital The explanation lies partly in the existence of local hospitals and partly in the relatively large distances to the hospital at Falun

Östersund (Z1) which like Växjo and Falun is a comparatively small town presents very high admission rates—up to age 80 no less than 7.0 per cent for males and 7.8 per cent for females For

the south-eastern part (Z3, in the province of Härjedalen) the figures are low, 1 0 per cent for both males and females from this area the DM patients generally go to the hospital at Sveg For the remainder of the admission area (Z2 the rural parts of the province of Jämtland) the figures are high viz. 3 9 and 6 4 per cent, in spite of the long distances to Östersund from certain parts of the area However, in view of the geographical features of Jamtland and the adjacent provinces the position of Östersund as the centre of the province is very marked It should be noticed in addition that the admission of DM patients in the advanced age groups is at a higher level at Östersund than at the other hospitals studied At least in part this difference may be regarded as a consequence of the circumstance just mentioned, a great many of the inhabitants in Jämtland have special reasons (other than disease) for visiting Östersund, whereas in respect of parts of the admission areas of the hospitals at Vanersborg Växjo and Falun there are ' competing towns (within or outside the admission areas)

Frequency of admissions

The total number of registrations (duration cards) is 7,385 However, for some patients there exist gaps in the series of registrations, these gaps may be due to migration (the patient being away from the area over a whole registration period), but for mild cases they may also be the result of a state of good control,

Table 13 Number of admissions and average annual frequency of admissions by sex, duration and age at onset

Sex	Duration	Age at onset						All ages	
		0–14 (J)		15–39 (A)		40– (L)			
		a	f	a	f	a	f	a	f
M	0– 4	1 492	5 0	3 044	3 9	5 703	3 1	10,239	3 5
	5– 9	884	1 7	1 303	1 4	2,192	1 5	4 379	1 5
	10–14	650	1 5	830	1.5	906	1 5	2,386	1 5
	15–19	421	1 5	657	1 6	457	1 7	1 535	1 6
	20–24	238	1 7	362	1 7	146	1 8	746	1 7
	25–	106	1 1	383	1 8	42	1 4	531	1 6
	Total	3 791	2 2	6,579	2.1	9 446	2.2	19 816	2 2
F	0– 4	1 534	5 8	2,201	4.2	9 107	3 2	12,842	3.5
	5– 9	1 067	2.3	1 126	1 7	4 039	1 7	6,232	1 7
	10–14	890	2.4	826	1 9	1 767	1 8	3 483	2 0
	15–19	494	2.3	565	2 0	714	1 9	1 773	2 0
	20–24	237	2.1	335	1 8	153	1 9	725	1 9
	25–	116	1 7	291	2.0	39	1 5	446	1 9
	Total	4 338	2.9	5 344	2 4	13 819	2.3	25 501	2 4

a = number of admissions
f = average annual number of admissions per patient

Table 14 Variations in admission frequency between hospitals and epochs

Hospital	Sex	Epoch				Total	
		E1 (1949)		E2 (1950)			
		a	*q*	*a*	*q*	*a*	*q*
E	M	2 490	0 86	2,854	0 93	5,344	0 89
	F	2,275	0 82	3 492	0 93	5 767	0 88
	M+F	4 765	0 84	6 346	0 93	11 111	0 89
G	M	2 273	1 16	1 909	1 12	4 182	1 14
	F	3 396	1 16	2,623	1 06	6 019	1 11
	M+F	5 669	1 16	4 532	1 08	10,201	1 13
W	M	2,446	1 27	3,239	1 05	5 685	1 13
	F	3 314	1 19	4 189	1 03	7 503	1 10
	M+F	5 760	1.22	7 428	1 04	13 188	1 11
Z	M	2,266	0 79	2,339	1 02	4 605	0 89
	F	2,982	0 84	3,230	1 01	6 212	0 92
	M+F	5,248	0 82	5 569	1 02	10 817	0 91
Total	M	9 475	0 98	10 341	1 02	19 816	*1.00*
	F	11 967	1 00	13 534	1 00	25 501	*1 00*
	M+F	21 442	0 99	23 875	1 01	45 317	1 00

a = number of admissions

q = quotient between observed and expected number of admissions expected number calculated from the overall admission frequencies by sex duration and age at onset

needing no hospital visits The total number of 5 year gaps is 250 and thus the *maximum* observation time spanned by our series would be 5 × (7 385 + 250) – 38,175 years for the 3,759 patients or about 10 years per patient

As may be seen from Table 6 the average time interval from onset to first admission is 2 3 years and on average it may be assumed that the last observation period for a patient does not cover more than 2 5 years Thus the *effective* observation time must be considerably shorter than 38 000 years in this connection it should be observed that the number of first admissions shows a marked increase with time An approximate calculation may be performed on the basis of the following assumptions

(a) For each patient, his first and last registration (duration card) is considered to represent 2 5 observation years

(b) If a patient is registered in one period only, the observation time is considered to be one third of the period, corresponding to the hypothesis that *times of entrance and exit are evenly* distributed and independent

(c) If there is a gap in the sequence of registrations the patient is considered to have left observation in the middle of the preceding period and to have again come under observation in the middle of the following period

The total observation time during the *duration period d will then* be determined through the formula

$$\frac{1}{5}t_d = c_d - \frac{1}{2}f_d - \frac{1}{2}l_d + \frac{1}{3}s_d - \frac{1}{2}(g_{d-1} + g_{d+1})$$

where

t = observation time in years
c = number of duration cards
f = number of first registrations
l = number of last registrations
s = number of single cards (first *and* last registration)
g = number of gaps (period missing)

Summing over all periods we obtain (using capital letters for the sums over all periods)

$$\frac{1}{5}T = C - P + \frac{1}{3}S - G$$

where P $(=F=L)$ is the number of patients

In this way, the total effective observation time may be assessed at 19 700 years or on average somewhat more than 5 years per patient

In Table 13 by dividing the number of admissions into the figures for the observation time the annual frequency of admissions is determined The data are given by sex age at onset and duration

The types by age at onset—juvenile early adult and late—represent about 17 27 and 56 per cent of the total time The lowest duration group 0–4 years covers about one half of the total observation time

The annual frequency of admissions shows a consistent picture according to duration, viz high figures during the first duration period and later 1 5 to 2 admissions per annum, during the first duration period the frequency of admissions is higher among juvenile diabetics than among diabetics with early adult onset and higher among these than among patients with late onset For the whole series the average annual frequency of admissions is 2 3

The variations in admission frequency between the four hospitals and the two epochs (–1949, 1950–) are shown in Table 14 The observed numbers of admissions have been divided into the expected numbers, viz those which would have obtained if the overall admission frequencies by sex duration and age at onset had been applicable throughout the whole material As will be seen from the table the variations by sex are very slight, the admission frequencies are higher at Vaxjo (G) and Falun (W) than at Vänersborg (P) and Östersund (Z) There has occurred a levelling out in so far as the quotients observed/expected are closer to unity in the later epoch (1950–) than they were in the earlier epoch (–1949)

CHAPTER III

Mortality among DM patients

Material and methods

One of the aims of this investigation has been to study the mortality of the DM patients In order to get a suitable series several difficulties had to be overcome In the first place we had to secure correct periods of observation Some of the physicians with the aim of providing the fullest possible data had taken the trouble to assemble earlier reports about their patients from other hospitals and from physicians outside the hospitals and included these reports in their own material However valuable this supplementary information may have been for the clinical study it had the drawback of introducing immortal persons into our material Obviously if these patients had died before their first visit to the investigation hospital in question, they would never have entered our series For this reason we have limited the mortality study to a period beginning in 1946 as we know that from this year onwards the immortality effect is negligible

For Växjo (G) the registration period ends with the year 1955, and we therefore decided to restrict the mortality study to the 10 year period 1946–55

Our preliminary examination of the punched cards showed an astonishing low number of registered deaths It was evident that the hospitals had not been able to register complete information about deaths among their diabetic patients For 1,440 out of the 3 759 patients we did not know for certain if they were living at the end of 1955 In the first place we asked the hospitals involved to complete their data as fully as possible In order to try to clear up the remaining uncertain cases we had to write a great many letters to the county registration offices and to the parishes where the patients had last been domiciled In this way we ultimately succeeded in getting almost complete information and in the total series no fewer than 464 previously ' unknown deaths were recorded

In all, our material for the period 1946–55 covers 160 male deaths in 7 498 observation years and 238 female deaths in 8 942 observation years To admit the subdivisions needed the material was treated in the following way For each sex the observation time was split according to calendar year duration in 5 year intervals and attained age in one year intervals The observation times were for every age multiplied by the corresponding death rate of the Swdishe population in the period 1946–55 (for

Table 15 Mortality of DM patients by sex and age 1946–55

Attained age	Number of observation years		Number of deaths				Death rate per thousand		Mortality ratio	
			M		F					
	M	F	O	E	O	E	M	F	M	F
0– 4	3	3	–	0 0	–	0 0	–	–	–	–
5– 9	41	40	–	0 0	–	0 0	–	–	–	–
10–14	196	200	–	0 1	–	0 1	–	–	–	–
15–19	430	417	–	0 5	1	0 3	–	2	–	3 6
20–24	497	448	1	0 8	6	0 4	2	13	1 2	14
25–29	527	365	3	0 8	5	0 4	6	14	3 6	13
30–34	596	340	5	1 1	5	0 4	8	15	4 7	12
35–39	562	360	1	1 2	–	0 7	2	–	0 8	–
40–44	586	410	4	1 7	2	1 0	7	5	2 3	2 0
45–49	591	527	3	2 8	3	2 0	5	6	1 1	1 5
50–54	648	727	7	4 8	9	4 3	11	12	1 5	2 1
55–59	619	975	16	7 4	14	8 8	26	15	2 2	1 6
60–64	689	1 225	18	12 0	26	17 9	26	21	1 5	1 5
65–69	625	1 226	26	18 2	53	30 1	42	44	1 4	1 8
70–74	492	969	30	23 9	50	41 3	61	52	1 3	1 2
75–79	289	527	30	22 9	41	38 3	104	78	1 3	1 1
80–84	99	141	15	12 5	17	16 8	159	121	1 2	1 0
85–89	7	42	1	1 4	6	7 8	143	143	0 7	0 8
90–	1	–	–	0 1	–	–	–	–	–	–
Total	7 498	8 942	160	112 2	238	170 6			1 4	1 4

O = Observed number of deaths

E = Expected number of deaths according to mortality in the general Swedish population 1946–55 (for males and females respectively)

males and females respectively) and these expected numbers of deaths were then added within 5 year intervals of age and compared with the observed numbers of deaths This means that if the diabetics had *strictly followed* the population mortality (and the population mortality had been stable over the 10 year period) the observed number of deaths should for every subdivision be equal to the calculated expected number As this quality is preserved under any additions we are entitled to make all combinations *needed The quotient* between the observed number of deaths and the number expected according to the population mortality is termed the *mortality ratio*

Mortality by age

The results of the calculation in respect of mortality by sex and age are shown in Table 15 The table gives, by 5 year age groups, the number of observation years within the period 1946–55 the observed and expected numbers of deaths the death rate for DM patients, and the mortality ratio Admittedly, the number of deaths is not very large but nevertheless the figures seem to give a good picture of the circumstances prevailing in a series of DM patients who have been admitted to specialized county hospitals in Sweden When evaluating the data it should not be overlooked that to a certain extent the existence of

Table 16 Mortality of DM patients by sex and calendar year 1946–55

Year	All durations				Duration 0–4 years			
	Number of deaths		Mortality ratio		Number of deaths		Mortality ratio	
	M	F	M	F	M	F	M	F
1946	10	14	2.0	2.3	2	6	1.1	2.4
1947	11	15	1.9	1.9	5	6	2.1	1.7
1948	9	12	1.3	1.3	5	4	1.6	0.9
1949	[illegible]	21	1.1	1.8	5	9	1.4	1.5
1950	16	16	1.7	1.1	7	9	1.6	1.2
1951	16	18	1.5	1.0	8	9	1.5	1.0
1952	23	34	1.8	1.6	12	6	2.0	0.6
1953	21	23	1.4	1.0	7	6	1.0	0.5
1954	19	48	1.0	1.7	8	23	1.1	1.8
1955	26	37	1.3	1.2	9	9	1.3	0.9
1946–50	55	78	1.6	1.6	24	34	1.6	1.4
1951–55	105	160	1.4	1.3	44	53	1.4	1.0
1946–55	160	238	1.4	1.4	68	87	1.4	1.1

DM has been revealed in connection with the patient s seeking medical advice for other diseases (intercurrent disease)

Adding over all ages we find a total mortality ratio of 160/112 2 = 1 43 for males and 238/170 6 = 1 39 for females When considering these figures one should bear in mind that the comparison with the population mortality is made for each sex separately Thus the total relative increase in mortality is almost identical for the two sexes

Mortality in separate years

The development of the treatment of diabetes during the last decades gives particular interest to the question whether there exists a trend in the mortality ratio during the period studied In order to throw light on this matter the total material has been divided simultaneously into calendar years 1946–55 and into 5 year duration periods For most combinations the expected number of deaths is too small to allow a statistical judgment and therefore the figures are given only for the combined durations and for the initial duration period 0–4 years (Table 16)

For the combined durations the mortality ratios oscillate around the total values with a decreasing tendency This supports the assumption that the mortality decrease among diabetics is parallel to the development in the general population Our material is too small however to permit a comparison of the rates of decrease

Mortality and duration

In view of the weak trend with regard to time we could study the influence of duration from the marginal figures of the above mentioned simultaneous table These data are shown in Table 17

There exists a tendency of a slight

Table 17 Mortality of DM patients by sex and duration 1946–55

Duration	Number of deaths		Mortality ratio	
	M	F	M	F
0– 4	68	87	1 4	1 1
5– 9	36	57	1 2	1 2
10–14	23	37	1 4	1 4
15–19	19	38	2 0	3 0
20–24	10	9	2 4	2 9
25–	4	10	1 1	5 6
Total	160	238	1 4	1 4

increase for males and a clear increase for females This result might be interpreted as supporting the pessimistic opinion that although modern treatment has succeeded in eliminating the initial risks combined with diabetes it has not been able to neutralize the effect of late complications especially those of renal type, which still seem to be a frequent consequence of diabetes However it is well known that diabetes varies much in severity and this makes it necessary to separate so far as possible classes of different severity In addition it should be remembered that in our series the great majority of cases with late onset belong to the shortest duration groups

Mortality and age at onset

If we look at the general mortality (Table 15) there is an obvious tendency for the mortality ratio to decrease with age Comparing, for instance, ages below 60 and ages 60 and over we find that the mortality ratio decreases from 1 9 to 1 3 for males and from 2 4 to 1 3 for females This could be the effect of different mortality ratios according to different ages of onset, which would agree with the general experience that cases of diabetes appearing early, especially before puberty, often have a bad prognosis whereas on the other hand a large number of old people show definite symptoms of diabetes without signs of complications and in many instances do not even have to use insulin For this reason we have studied our material according to age at onset Since in the mortality study the exact age of onset was not included we have used approximate classes constructed from 5 year intervals of attained age x to $x+4$, and 5 year intervals of duration d to $d+4$ which make up an interval for age at onset from $x-d-4$ to $x-d+4$ Within this

Table 18 Mortality of DM patients by sex and age at onset 1946–55

Age at onset	Duration	Number of deaths		Mortality ratio		
		M	F	M	F	M+F
0 19	All	7	14	3 2	11 7	6 2
15–44	All	11	20	1 1	3 1	1 9
40–	0– 4	66	85	1 4	1 1	1 2
	5– 9	36	57	1 3	1 2	1 2
	10–14	18	28	1 3	1 1	1 2
	15–	22	34	1 8	2 5	2 2
	All	142	204	1 4	1 3	1 3
Total		160	238	1 4	1 4	1 4

interval we get a triangular distribution with the mean $x-d$ the consecutive intervals overlap The results are shown in Table 18

Although the expected number of deaths for the juvenile and early adult types of diabetes is small the clear picture emerges that juvenile diabetes shows an impressively high mortality ratio, whereas late diabetes containing cases with onset at age 40 or over shows a mortality ratio of about 1 3 For this latter type a division is made according to duration, too As can be seen from the table the mortality ratio is around 1 2 for durations below 15 years but higher or on average 2 2, for durations 15 years or over It may be noted that the high mortality ratio of the juvenile diabetics only slighty affects general mortality, because of the comparatively low prevalence of the disease and the low general mortality in the actual ages In other words the high mortality ratio at younger ages does not imply a high absolute mortality

Inferences from the mortality study of DM patients

Compared with the population mortality the diabetics in our hospital series show a mortality ratio of about 1 4 (all ages taken together) For juvenile dia betics the mortality ratio is considerably higher (order of magnitude 6 0) and for diabetics with age at onset 40 or over it is about 1 3 For late diabetes the mortality ratios are about 1 2 for durations below 15 years and about 2.2 for durations 15 years or over

It should be remembered, however that selective factors, in particular the detection of DM in connection with other diseases may influence the results of statistics on mortality of DM patients who have been admitted to hospital On the other hand it should be taken into account that there may occur a certain selection of another kind, inasmuch as severely disabled persons may be under-represented in a general hospital series Nevertheless, the results indicate that the excess mortality of diabetics in general is less than is usually considered to be the case These problems will be further discussed in Chapters IV and VIII

There are a great many investigations concerning the mortality of DM patients based mostly on hospital series and insurance material On the whole, they show a higher mortality, and even a higher excess mortality in comparison with the general population than has been found in the present series On the other hand the sex and age pattern of the excess mortality in our series seems to agree fairly well with other statistics It would fall outside the scope of the present study to discuss these statistics and here we will only make a reference to a recent study from Birmingham by Hayward and Lucena (1965) and the very interesting discussion of their paper

CHAPTER IV

Mortality and morbidity risks for DM and its prevalence in Sweden

General aspects

In view of the varying conditions obtaining in the registration of new cases of DM it is very difficult to arrive at a definite assessment of the morbidity risks for DM at different ages—even if these risks do not change with time As is well known from screening procedures and other inventories, and as will be shown in greater detail in a later section, at a given point of time there will generally be at least as many instances of manifest DM that are undiagnosed as there are instances of diagnosed ' DM Here the term ' diagnosed is taken to mean persons who have been told by a physician that they have diabetes and the term undiagnosed to mean persons who have manifest diabetes but do not know it (cf Fisher & Vavra 1964)

Of course, circumstances are by no means the same all over the world, and even within one country they may vary considerably between different areas, there are a great many factors of importance with regard to the dividing line between diagnosed and undiagnosed instances of DM, and it is not to be expected that this boundary will not change with time So far as Sweden is concerned, some of these factors are as follows (in several respects the situation is fairly similar in most other developed countries)

1 Both absolutely and relatively the number of doctors and the number of beds in hospitals have steadily increased

2 Laboratory tests of urine and blood have become routine procedures

3 Because of rising standards and improved communications, visits to doctors and at hospitals have become more frequent

4 In the compulsory health insurance scheme introduced in 1955, a doctor s certificate is required when claims are made in respect of a period of illness exceeding seven days

5 The prevalence of DM, and hence the general knowledge of the disease has gradually increased, owing partly to progress in therapy (directly, through insulin and better dietetic treatment indirectly through new methods for preventing and curing other diseases by means of sulpha preparations antibiotics antihypertensive drugs etc), and partly to the general decline in mortality and the altered age composition of the population

6 In several districts systematic detection campaigns have been performed (on a more or less general basis) routine controls are made in connection with the preventive maternity welfare activities, and regular health control is becoming more frequent (in particular for employees over say, 40 years of age)

This list could easily be greatly extended but the examples given will probably be sufficient to show that a registered increase with time of the prevalence or incidence of diagnosed DM (in a certain group by sex and age) cannot—at least without thorough analysis—be taken as evidence of an increasing prevalence or incidence of DM in the population (in this group by sex and age) Naturally, the *overall* prevalence of DM will increase as a consequence of the displacement upwards of the age composition of the population this effect will be accentuated if the relative excess mortality of diabetics is becoming weaker In the same way it is obvious that the *overall* incidence of DM will increase, since the morbidity risk for DM increases markedly with age

Nevertheless whatever the situation in regard to diagnosed and undiagnosed instances of DM may be at a given point of time, many of the undiagnosed cases then existing will be diagnosed later on either because the disease itself causes the afflicted person to go to a hospital or to consult a doctor outside the hospitals or because the person in question becomes afflicted with other diseases and on seeking medical aid is found to have DM This latter situation will be more common

(a) when it becomes more frequent that diseased persons seek medical aid,

(b) when it becomes more frequent that tests of urine and blood are analysed

(c) where doctors have sufficient time to investigate the patient s symptoms etc ,

(d) where doctors have contacts with the family members of DM patients and inquire into their state of health and if it is called for, test them with regard to the possible presence of DM

As a rule, the knowledge concerning the existence of chronic diseases is greatest at the latest possible point in the person s medical history i e immediately before or immediately after his death (before, since the doctor can then have the patient s own description of symptoms and can perform clinical examinations laboratory tests etc , after, since there may be an autopsy)

The recording of deaths is always more complete than is the recording of various forms of disease As a rule the available statistical data on mortality are considerably more accurate than are the available statistical data on morbidity Generally speaking both the knowledge of a disease and the registration of a disease become more complete and accurate as the individual grows older At any rate this must be true as regards chronic diseases and very malignant diseases In both these cases a careful scrutiny of mortality statistics may prove to be the most efficient method for arriving at conclusive results concerning the morbidity

For quite different reasons *diabetes mellitus* and *cerebrovascular disease* seem

to be especially promising topics, where the study of *mortality* is intended to throw light on *morbidity* (Larsson 1967)

Diabetes mellitus may be regarded as a permanent disease recovery is very rare (if it occurs at all) On the other hand, apart from instances of very early onset, DM *per se* can hardly be regarded as an ultimate cause of death, although it is often registered as the primary (underlying) cause Where the existence of DM is known to the certifying doctor, the conditions in Sweden (and the traditions and training of Swedish doctors) will guarantee that the presence of DM will be stated in the death certificate (as the primary cause or more often, as a contributory cause) [1]

Cerebrovascular disease (stroke apoplectic insult) is as a rule easily recognizable even by laymen The morbidity risks for cerebral haemorrhage as well as for cerebral embolism and thrombosis are very low at younger ages in the age group 50–54 the death rate for cerebrovascular disease (primary cause contributory cause or complication) is about 0 5 per thousand The morbidity risk increases heavily with age The outcome is often fatal and if the patient recovers (fully or partly) there may in many cases occur one or more further insults Even where such a recurrence does not take place it may be assumed that the previous attack will as a rule be reported in the death certificate as a contributory cause thus in this respect the occurrence of an apoplectic insult may be regarded as establishing a permanent situation

Hence in the main, it can be assumed that the Swedish statistical data on causes of death are much more complete and reliable in respect of both clinically diagnosed diabetes mellitus and cerebrovascular disease than in respect of most other diseases, *provided that not only the primary cause but also contributory causes and complications are included in the statistics* The character of these diseases will make it possible—in different ways—to perform an assessment of the morbidity risks by sex and age on the basis of mortality statistics Especially for the older age groups, such indirect determination seems to be considerably more reliable than direct determination on the basis of morbidity statistics of different kinds (hospital and health insurance data, cross section inventories, screening procedures, questioning of physicians, statistics on insulin consumption)

The main object of the present chapter is to investigate the homogeneity and general characteristics of the recording of deaths from DM, and to perform an assessment of the morbidity risks for DM by sex and age [2] In Chapter IX, some comments will be made on the conclusions that can be drawn with regard to the importance of genetic factors for the aetiology of DM [3]

[1] Where the certifying doctor and his colleagues did not interview the deceased person and do not have any contacts with the family members there may of course be a gap in the registration of diagnosed DM even if an autopsy is performed As will be seen in what follows this situation will occur mainly in the largest cities and in respect of sudden death (accidents acute illness suicide) especially for persons dying outside their district of domicile

[2] For convenience the expression *death from DM* is used to cover all cases where DM is stated as the primary cause of death or as a contributory cause

[3] A parallel study has been made in respect of cerebrovascular disease Tage Larsson Mortality from cerebrovascular disease in *Stroke Thule International Symposia* Nordiska Bokhandelns Forlag Stockholm 1967 pp 15 40 For comparison some data from this study will be included in the tables

The material

Since 1951 the statistics on causes of death in Sweden have been presented in accordance with the W H O scheme *Manual of the international statistical classification of diseases, injuries and causes of death* (Geneva 1948 and 1949) Certain amendments have been adopted, in particular from 1960 with regard to the delimitation between accidents and disease when stating the primary (underlying) cause of death (cf Larsson 1965, p 69) As a rule the data given in the present chapter refer to the Intermediate List (the A list)

There are three categories of forms used for reports on causes of death viz certificate (*dödsbevis*), notification (*dödsorsaksavi*) and report from abroad (*dödsorsaksuppgift*)

The certificate signed by a physician, is compulsory for deaths occurring in urban areas, for deaths in rural districts a certificate is issued if a doctor has attended the deceased during his last illness or has examined the body after death

Up to 1960 the statistics dealt solely with *primary (underlying) cause* Since 1961 however, the Central Bureau of Statistics has made a registration of *complications* and *contributory causes of death* in addition to the primary cause the tape records include a maximum of 3 complications and a maximum of 4 contributory causes (stated according to the detailed W H O list) further, there are registered date of birth sex, marital status domicile date of death character of report (certificate etc) and whether an autopsy was performed or not

For the present study of mortality from diabetes mellitus (and the parallel study of mortality from cerebrovascular disease) a special processing of these tape records for the years 1961–63 was performed [1] The scope of this material is shown in Table 19 it covers 10 597 deaths from DM (primary or contributory cause, 4 379 males and 6,238 females) or 4 7 per cent of the total number of deaths in 1961–63 (males 3 6 per cent, females 5 9 per cent) Otherwise all data given in the following sections are based on published official statistics

During the period 1961–63 there occurred in Sweden 226,806 deaths (120 462 males 106 344 females) The statistics on causes of death are based on certificates for 97 64 per cent of these deaths (notifications 2 04 per cent reports from abroad 0 32 per cent) In 35 4 per cent of all deaths an autopsy was performed

The available information on causes of death has steadily become more complete Thus for the period 1961–63 the group 'symptoms senility and ill-defined conditions (XVI) contains no more than 1 31 per cent of all deaths

As will be seen from Table 19, during the period 1961–63 there were registered 3 324 deaths with DM as the primary cause and no fewer than 7,273 deaths with DM as a contributory cause In view of the nature of DM it is evident from these figures alone that statistics

[1] For reasons of economy in this processing the *age* at death was calculated as the difference between the calendar year of death and the calendar year of birth Where the exact age is rounded off downwards the expression *attained age* is used

Table 19 Deaths from DM in Sweden 1961–63

Cause of death		Primary cause		Contributory cause		Total		
		M	F	M	F	M	F	M+F
Number of deaths		120 462	106 344			120 462	106 344	226 806
Cause unknown	XVI	1 277	1 704			1 277	1 704	2 981
Diabetes	A63 = 260	1 419	1 905	2 940	4 333	4 359	6 238	10 597
Diabetic coma	260 1	93	129	11	16	104	145	249
Gangrene	260 4	235	296	93	123	328	419	747
Nephropathy without hypertonia	260 5	181	212	36	56	217	268	485
Other specified		114*	134*	37	54	151	188	339
Not specified	260 0	796	1 134	2 763	4 084	3 559	5 218	8 777

* Other specified (primary cause)

Hyperglycaemic coma (260 2)	9 M	+ 7 F
Acidosis (260 3)	6	+ 4
Nephropathy with hypertonia (260 6)	60	+ 61
Retinopathy (260 7)	20	+ 37
Cataract (260 8)	0	+ 1
Other complications (260 9)	19	+ 24

which are based exclusively on primary causes of death must be treated with the utmost caution

Where DM is stated as the primary cause complications are specified (i e given with codes 260 1–260 9) for 42 per cent of the deaths where DM is stated as a contributory cause, complications are specified for only 7 per cent of the deaths Taking primary and contributory causes together we find that complications are specified for no more than 17 per cent of the deaths Hence, it can be concluded that on the whole the information in the death certificates concerning complications must be very incomplete [1]

[1] It should be noticed however that the actual situation is far better than is indicated by the adduced percentages Where unspecified DM (260 0) is *given as the primary cause of* death there are often statements about one or more contributory causes where unspecified DM is given as a contributory cause there is always information on the primary cause and to a not inconsiderable extent about other contributory causes

Interest should be focused on the total (DM as the primary or a contributory *cause) and not on the subgroups*

Statistics on mortality from DM

Certain statistical data on mortality from DM in Sweden will be presented in the following tables (Tables 21–31) To a great extent these tables speak for themselves, and therefore the comments given here can be kept very brief (for general comments, cf Larsson 1965)

Total mortality

As a background Table 20 shows the general death rates by sex and age for 5 year periods 1946–65 for the 3 year period 1961–63, and for the separate years 1956–65 Seen against the trend, mortality was comparatively high in 1962 and comparatively low in 1961 and

Table 20 General death rates by sex and age 1946–65

Sex and year	Death rate per 10 000 of the average populat on in age group (atta ed age)																		
	0*	1 2	3 4	5 9	10–14	15 [illegible]	0 24	25 29	30 34	35 39	40–44	45 49	[illegible] 54	55 59	60–64	65 69	70 74	75 79	80
Males																			
1946–50	270	19	12	8	6	12	19	[illegible]	[illegible]	23	31	49	78	122	195	314	505	850	1 800
1951–55	218	15	9	7	5	10	13	13	15	20	27	44	69	116	185	298	489	814	1 724
1956–60	190	13	8	6	4	10	11	12	14	18	25	40	65	110	183	297	489	807	1 673
1961–65	172	10	6	5	4	9	11	11	13	[illegible]	24	39	65	108	184	299	492	811	1 661
1961–63	176	11	6	5	4	9	11	11	13	17	24	39	64	108	186	298	491	815	1 676
1956	190	14	9	6	4	10	12	13	16	18	27	42	69	113	186	295	486	803	1 684
1957	199	14	9	6	5	10	11	13	15	18	26	42	71	114	193	303	487	833	1 727
1958	181	12	9	6	4	10	10	11	13	[illegible]	25	37	63	106	181	297	478	792	1 656
1959	192	12	8	6	4	9	12	12	13	17	23	41	63	105	167	285	483	783	1 625
1960	189	11	8	5	4	9	11	11	13	17	26	39	62	114	187	305	511	822	1 679
1961	177	11	7	5	4	9	10	11	14	17	23	37	65	108	182	297	487	786	1 626
1962	176	11	6	5	4	9	11	11	12	17	26	39	66	108	188	306	500	846	1 702
1963	174	10	5	5	4	10	12	11	13	17	23	40	62	109	187	292	485	814	1 700
1964	159	7	7	5	4	10	12	11	13	19	25	40	64	112	178	302	487	800	1 628
1965	149	9	6	5	4	9	11	11	14	20	25	38	68	104	183	296	499	809	1 649
Females																			
1946–50	208	15	9	5	5	8	12	13	16	21	27	41	63	95	154	259	456	786	1 719
1951–55	167	13	6	4	3	5	6	8	11	14	22	34	54	84	137	237	423	734	1 637
1956–60	145	10	5	3	3	4	5	6	8	12	19	30	46	72	122	214	391	693	1 540
1961–65	132	7	5	3	3	4	4	6	8	11	17	28	43	65	108	192	351	642	1 464
1961–63	133	7	5	4	3	4	4	6	7	11	17	28	43	67	109	196	360	658	1 502
1956	155	11	5	3	3	5	5	6	9	13	19	30	47	74	124	225	401	701	1,547
1957	155	9	5	4	3	4	5	6	9	12	19	30	47	77	130	223	405	718	1 606
1958	136	10	6	3	3	4	4	6	8	13	20	31	46	73	122	213	384	689	1 530
1959	138	10	4	3	2	4	5	6	7	12	19	27	44	69	117	206	375	664	1 485
1960	142	10	6	3	3	4	5	7	8	12	18	31	46	70	117	204	390	694	1 537
1961	137	7	6	4	3	4	5	6	7	10	16	30	44	67	111	194	368	665	1 490
1962	131	8	5	4	3	4	4	6	7	11	18	26	43	66	110	195	369	664	1 542
1963	132	7	5	3	3	5	5	6	8	[illegible]	16	29	41	67	107	198	345	646	1 474
1964	124	7	4	3	2	4	5	6	8	12	17	27	43	64	111	188	342	622	1 407
1965	121	7	4	3	2	4	4	5	8	11	18	27	42	64	105	186	334	615	1 419

* Deaths under 1 year of age per 10 000 live births

1963, together, the three years 1961–63 may be said to represent a 'normal' level for mortality

Mortality from DM in the whole country 1961–63

Table 21 gives—by sex and 5 year age groups—the average population 1961–63, the total number of deaths and the numbers of deaths from unknown cause and from the primary cause DM (260=A63=Dp, with separate data on the sub group 'not specified', 260 0), further, the shares of deaths from unknown cause and from DM (primary cause) are stated At the bottom of the table and in Fig 6 the shares of deaths from DM—primary cause as well as total—are shown [1] The absolute numbers of deaths from DM by sex and age are given in Table 23

Both for males and for females, the share of deaths from DM (primary as well as contributory cause Dt/T) at younger ages rises markedly with age to reach a peak of about 5 per cent for males and about 8 per cent for females in the age groups 30–34 and 25–29 respectively then there is a marked decrease to a minimum of about 2 per cent in the age group 45–49 and after that a fairly even rise to a new maximum of nearly 6 per cent for males and nearly 9 per cent for females in the age groups 75–79 and 70–74 In the most advanced ages there is again a fairly even decrease, to a share below 2 per cent in the age group 90 and over The variation of the death shares gives an impression that there exists a certain gap in the registration of DM among deaths in the most advanced age groups (an *age gap*), the high shares at younger ages reflect an existing excess mortality among persons afflicted with DM (cf p 62)

In Table 22 death rates by sex and age 1961–63 for different groups of causes are calculated At the bottom of the table the death rates for DM—primary cause as well as total—are shown [2]

The death rates for DM (Dt/P) by sex and age are illustrated in Fig 7, in addition there are shown the total death rates (irrespective of cause, T/P) in the younger age groups and, for comparison the death rates for violence (accidents and suicide) [3] Up to age 60, mortality from DM is higher for males than for females, in the age interval 60–90 it is lower for males than for females Up to age 65 for the males, and up to age 55 for the females, mortality from DM is far below mortality from violence At age 70, mortality from DM is of the same order of magnitude as the total mortality at ages about 35 for males and about 45 for females Before 60, mor

[1] Account has been taken of the gaps arising from the registration of deaths as due to unknown cause (XVI) It should be noticed that the data are grouped by *age* (not by attained age) and are given *per 10 000 deaths* (not per 1 000 deaths)

[2] Account has been taken of the gaps due to deaths from unknown cause (XVI) but otherwise the figures are wholly based on *uncorrected* data for the whole country the implications of geographical differences in respect of recording (and, possibly morbidity from DM) will be discussed in a later section The data refer to attained age and are given per 1 000 000 of the average population in the group (exaggerated exactitude)

[3] The figures concerning violent deaths refer to primary cause alone but according to the rules adopted in 1960 violence will generally be recorded as the primary cause of death and not as a contributory cause (cf Table 31A p 103)

Table 21 Population total deaths, deaths from DM and death shares by sex and age 1961–63

Average population Cause of death	Sex	0 003 × average population number of deaths and share of deaths in age group (attained age)																		
		All ages	0- 4	5- 9	10 14	15- 19	0- .4	25- 9	30 34	35 39	40 44	45- 49	50 54	55- 59	60 64	65 69	70 74	75 79	80 84	85 89	90
3 × average population P thousands	M	11 318	800	819	894	963	754	668	694	769	828	794	794	696	591	471	351	241	129	50	12
	F	11 368	757	773	[illegible]	[illegible]	730	649	686	758	814	783	788	721	636	534	422	295	163	66	19
Number of deaths primary cause																					
All causes T	M	120 462	3 476	415	340	866	833	738	894	1,316	1 968	3 083	5 084	7 551	10 974	14 065	17 213	19 619	17 366	10 572	4 089
	F	106 344	2 487	272	239	366	318	391	508	797	1 353	2 204	3 370	4,799	6 928	10 456	15 194	19 440	19 072	12 365	5,785
Unknown cause XVI	M	1 277	6	–	1	4	7	2	9	8	11	16	17	17	33	43	64	136	233	332	338
	F	1,704	5	1	1	2	3	1	2	2	6	7	9	7	16	27	73	144	358	479	559
DM 260=A63=Dp	M	1,419	–	–	3	4	11	35	38	[illegible]	48	32	73	97	132	169	214	247	182	81	15
	F	1 905	–	2	1	4	9	28	30	19	26	19	45	70	162	283	389	418	257	122	21
DM not specified 260 0	M	796	–	–	1	2	2	14	10	11	11	11	34	51	78	108	129	157	111	56	10
	F	1,134	–	2	–	1	1	5	4	6	13	9	17	31	83	172	254	268	171	[illegible]	13
Percentage of 260 = Dp																					
DM not specified 260 0	M	56	–	–	33	50	18	40	26	29	23	34	47	53	59	64	60	64	61	69	67
	F	[illegible]	–	100	0	25	11	18	13	32	50	37	38	44	51	61	65	64	67	69	62
Per 1 000 deaths																					
Unknown cause XVI	M	11	2	–	3	5	8	3	10	6	6	5	3	2	3	3	4	7	13	31	[illegible]
	F	16	2	4	4	5	9	3	4	4	4	3	3	1	2	3	5	7	19	39	97
DM 260=A63=Dp	M	12	–	–	9	5	13	47	43	29	24	10	14	13	12	12	12	13	10	8	4
	F	18	–	7	4	11	[illegible]	72	59	24	19	9	13	15	23	27	26	22	13	10	4
*DM per 10 000 deaths (known causes) groups by age**																					
Primary cause Dp	M	119	–	–	93	49	104	420	480	317	236	113	136	134	121	123	126	127	106	[illegible]	36
	F	182	–	36	85	114	253	695	628	250	201	[illegible]	140	133	221	278	259	226	140	106	43
Primary or contributory cause Dt	M	166	–	–	93	62	116	490	515	381	326	206	273	310	345	408	446	478	421	286	168
	F	596	–	108	85	142	253	802	688	[illegible]	[illegible]	171	256	368	612	790	886	794	607	427	174

* Deaths from unknown cause (XVI) have been proportionately distributed over known causes within their group by sex and age

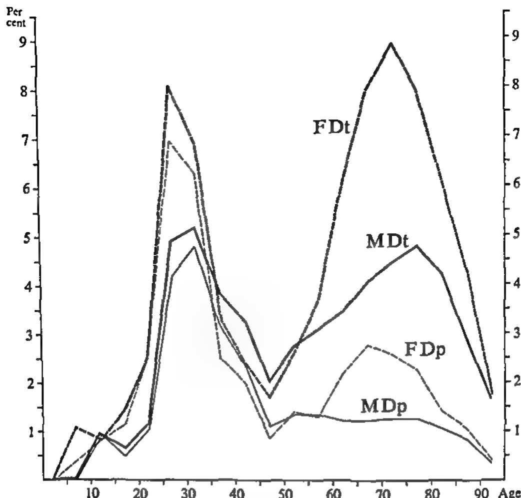


Fig 6 Shares of deaths from DM by sex and age 1961-63

tality from DM is below 0 4 per thousand and before 50 it is below 0 1 per thousand At younger ages, the death shares from DM, as shown in Fig 6, are large not because mortality from DM is high but because total mortality is low

In particular for deaths occurring at advanced ages the certifying doctor has a certain choice when stating the causes of death very often the deceased person may have been afflicted with two or more different diseases and opinions concerning the importance of the patient s disease history' may vary In addition the doctor s knowledge of this disease history may be more or less complete (for instance depending on whether he has been the patient s family doctor , whether he has examined the patient, whether he has interviewed family members, etc) Also, the post mortem examination may be more or less penetrating (inspection only,

Table 22 Death rates for different causes by sex and age 1961–63

Cause of death		Sex	All ages	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90
			Death rate per 100 000 of the average population in age group (attained age)																			
All causes		M	1 064	435	51	38	90	110	110	129	171	238	388	640	1 084	1 858	2 984	4 908	8 154	13 440	21 161	34 434
		F	935	329	35	28	40	44	[illegible]	74	105	166	281	427	666	1 089	1 958	3 603	6 582	11 670	18 841	30 712
Primary cause																						
Infect	I	M	12	5	1	1	2	2	1	4	5	7	9	14	18	28	33	50	65	65	110	147
		F	7	5	1	1	1	1	2	2	3	4	5	6	9	11	17	27	[illegible]	32	62	82
Neopl	II	M	206	13	7	7	10	11	15	22	28	51	[illegible]	152	274	472	759	1 088	1 531	2 164	2 389	2 405
		F	190	9	8	7	7	8	14	22	43	76	136	195	281	386	549	750	1 025	1 408	1 693	1 792
Diab		M	13			0	0	1	5	6	5	5	4	8	14	22	36	61	103	143	166	138
260 = A63 = Dp		F	17		0	0	0	1	4	5	3	3	2	6	10	25	54	92	143	160	193	124
Nerv	VI	M	132	10	2	2	3	2	4	7	12	16	27	53	94	171	341	681	1 281	2 203	3 356	4 902
		F	153	8	2	2	2	3	4	7	8	14	28	52	80	145	317	660	1 265	2 240	3 297	4 795
Circ	VII	M	433	1	1	1	3	3	5	9	24	45	105	217	427	802	1 300	2 174	3 708	6 236	10 551	18 599
		F	371	1	1	2	2	2	6	6	9	19	43	73	159	338	698	1 486	2 976	5 617	9 912	17 366
Resp	VIII	M	58	24	3	2	2	2	2	3	3	6	8	16	29	64	105	244	502	1 068	2 077	4 241
		F	56	20	3	1	3	1	2	3	3	4	5	9	16	30	77	179	440	945	1 694	3 309
Digest	IX	M	44	16	1	0	2	2	2	6	9	13	22	31	55	86	130	213	316	497	672	1 010
		F	41	11	1	2	2	3	[illegible]	3	5	6	10	20	30	50	90	172	297	521	707	976
Gen urin	X	M	36	1	1	1	2	3	5	3	9	8	14	19	29	46	77	168	312	586	938	1 570
		F	21	1	1	1	1	2	3	3	7	9	14	19	26	36	53	79	115	192	236	394
Oth dis		M	42	335	5	5	7	3	3	4	4	8	15	21	28	38	53	67	104	132	274	385
		F	38	255	6	3	5	6	6	5	5	8	12	16	24	31	48	77	107	168	270	399
Violence		M	88	30	30	19	59	81	68	65	72	79	99	109	117	129	148	162	232	346	628	1 037
XVII + XVIII		F	41	19	12	9	17	17	17	18	19	23	26	31	31	37	55	81	174	387	777	1 475
*Diabetes per 1 000 000 of the average population**																						
Primary		M	127			3	4	12	45	59	52	55	42	[illegible]	141	216	358	609	1 027	1 436	1 883	1 343
cause	Dp	F	170		1	[illegible]	4	11	40	45	25	32	23	57	85	231	525	897	1 461	1 610	2 073	1 382
Primary or		M	389			3	5	13	52	63	61	75	76	165	326	615	1 191	2 140	3 881	5 699	6 327	6 233
contributory cause	Dt	F	557	–	4	2	5	11	46	49	33	38	45	106	234	641	1 488	3 069	5 127	7 014	8 328	5 590

* Calculated from distribution by *age* but not otherwise graduated. Throughout, deaths from unknown cause (XVI) have been proportionately distributed over known causes within their group by sex and age.

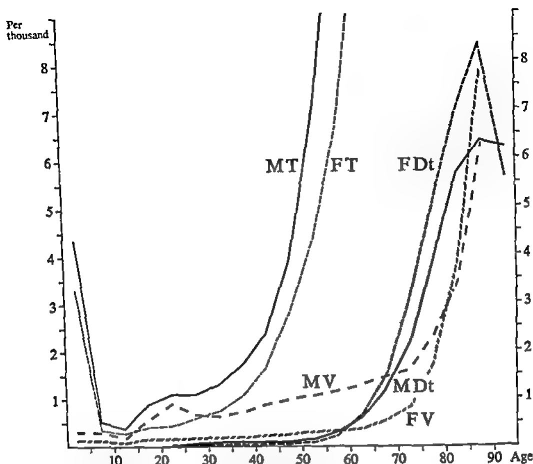


Fig 7 Death rates for DM (Dt) violence (V) and all causes (T) by sex and age 1961–63

autopsy macroscopic or microscopic brain examination) even where an autopsy is performed by a trained pathologist there may be a choice in respect of the patho anatomical diagnosis for instance one connected with the skill and the special interests of the pathologist in question

However provided that the occurrence of DM is mentioned in the certificate this choice does not involve any real obstacles in the present connection Of course, the opinion of the certifying doctor may influence the classification of the disease—i e whether it is recorded as the primary cause or as a contributory cause—and hence there may arise certain types of bias in the statistics on *primary* causes of death [1] In the processing of *both* primary and contributory causes the only relevant source of error is *omission*, arising either because the doctor (or other informant) is unaware

[1] It should be mentioned that a thorough scrutiny and revision of the certificates is made by the Central Bureau of Statistics

of the occurrence of DM or because his statements do not contain sufficient information[1] The general characteristics of DM, and the conditions obtaining in Sweden, make it rather unlikely that such omission will occur to any considerable extent[2]

Lombard and Joslin (1958) investigated the death certificates for 1 000 DM patients, diagnosed at the Joslin Clinic who died in 1950–57 For 33 per cent DM was recorded as the underlying cause of death for 44 per cent DM was otherwise registered and for 23 per cent it was not mentioned at all The authors make the following general statements which are of great interest in the present connection

Studies of long term trends of death rates were made more difficult by the adoption in 1948 of the sixth revision of the *Manual of the International Statistical Classification of Diseases Injuries and Causes of Death* The earlier edition of the *Manual* gave priority to certain causes of death if they appeared on the death certificate The sixth revision takes as the cause to be coded the underlying cause of death as stated by the certifying physician irrespective of the causes that previously had priority

Formerly if a certificate mentioned both diabetes and coronary artery disease the cause of death was always tabulated as a death from diabetes whereas a certificate that mentioned cancer and diabetes was always called a cancer death Since the adoption of the sixth revision the position of the diseases on the death certificate determines which one will be called the underlying cause of death As a result of this new classification the number of registered deaths from diabetes decreased appreciably

It is probable that the sixth classification is far superior to the earlier one for indicating the underlying causes of death but unless tables are published showing secondary causes of death also a study of diabetes from the death records can be misleading

The authors conclude

The reported number of deaths from diabetes represents approximately a third of the number of persons dying with the disease Nearly a quarter of those dying with the disease do not have the word diabetes on their death certificates either as an underlying cause of death or as an other significant condition'

It is evident that diabetes morbidity cannot be well estimated from the death records that long term trends can be misleading and that a part of the increase in the number of deaths from coronary artery disease is apparent rather than real

The students of the disease would be benefited greatly if physicians would include diabetes as one of the conditions present at the time of death and if the compilers of statistics from death records would make it a routine matter to publish combined causes of death

Possible gaps in the registration of DM

The problems may be formulated as follows Let us denote (in groups by sex age domicile, etc)

[1] The third possibility viz. that the occurrence is entered in the certificate but is not included among the data in the tape records is not applicable in respect of DM

[2] According to estimates made by Joslin (Joslin *et al* 1959) for 28 per cent of all his diabetic patients in the U S A diabetes was not mentioned anywhere in the death certificates Lancaster and Maddox (cf statement by J Mallins *J Inst Actuaries 91 3* 1965 p 327 discussion of the paper by Hayward & Lucena) reckoned with regard to their diabetic patients in Australia that for 33 per cent of the males and 50 per cent of the females diabetes was not mentioned at all in the death certificates However as already mentioned the tradition and training of Swedish doctors will guarantee that the omissions are at a lower level in Sweden — Certain data from Oslo recently given by Westlund (1966) will be discussed in a later connection (p 96)

Dp = deaths with DM registered as the *primary* cause

Dc = deaths with DM registered as a *contributory* cause

Dt = Dp + Dc = *total registered* deaths with DM registered as a cause of death

Dn = deaths where the deceased person was afflicted with DM but the disease was *not registered* as a cause of death

Dm = Dt + Dn = *all* deaths with DM

In part, the *completeness* of the data can be elucidated by means of a study of the connections between certain series by sex and age, viz the frequency to which registered DM is recorded as the primary cause of death (the quotients Dp/Dt) the frequency of autopsies (the quotients Da/D) the geographical differences (the quotients O/E where O is the observed number and E is the expected number calculated on the hypothesis of geographical homogeneity of the figures by sex and age) and the frequency to which DM is registered as a contributory cause for different primary causes of death

Is it possible by scrutinizing the variations of the data mentioned above for the known series Dp Dc and Dt, to arrive at conclusions concerning the magnitude of Dn and Dm?

In so far as current views concerning the prevalence of DM are based on mortality statistics they have been based solely (or at least mainly) on those concerning Dp However we know that among deaths in 1961–63 the overall quotient Dp/Dt is no more than 0 314 and, further that this quotient varies markedly by sex age domicile, etc Therefore it is obvious, as already stressed, that conclusions based on data concerning Dp must be made with great caution, in fact, it is only where surprisingly high figures (for absolute numbers death shares or death rates) are found that sure conclusions can be drawn

Can the possible "skewness (the variability by sex, age domicile, etc) of the quotients Dt/Dm be evaluated when the skewness of the quotients Dp/Dt is known?

Can the probable gaps in the registration be evaluated, for instance in the form Dm/Dt, hence the factor by which the observed figures are to be multiplied, or in the form Dm – Dt, hence the additions to be made to the observed numbers? Can there be stated minimum or maximum limits (or both) for these factors or additions?

The gaps may vary with sex age, domicile cause of death etc In the following analysis the evaluations will be made separately for each sex For convenience, the possible gaps may be systematized in five categories, viz

(a) *age gaps*, connected with variations of the completeness of the registration by age,

(b) *cause gaps*, connected with incomplete registration of DM as a contributory cause for certain types of primary cause of death

(c) *domicile gaps* connected with geographical variations (by county between rural and urban areas, etc) in the completeness of the registration,

(d) *time gaps*, connected with a possible underregistration during the period 1961–63 and

Table 23 Number of deaths from diabetes and cerebrovascular disease, percentage primary diagnosis, and percentage autopsies by sex and age 1961–63

	Sex	All ages	0 4	5 9	10 14	15 19	[illegible]0 [illegible]4	25 29	30 34	35 [illegible]	40-44	45-49	50 54	55 59	60-64	65 69	70 74	75 79	[illegible] 84	[illegible] 89	90
Number of deaths																					
Dp	M	1 419	–	–	3	4	9	30	41	40	45	33	65	98	127	169	213	247	[illegible]	94	16
	F	1 905	–	1	[illegible]	4	8	26	31	19	26	[illegible]	46	61	147	280	379	431	264	136	26
Dc	M	2 940	–	–	–	1	1	5	3	8	17	27	66	129	236	392	538	686	551	222	58
	F	4 333	–	2	–	1	–	4	3	6	5	17	38	108	261	515	916	1 084	883	411	79
Dt	M	4 359	–	–	3	5	10	35	44	48	62	60	131	227	363	561	751	933	736	316	74
	F	6 238	–	3	2	5	8	30	34	25	31	35	84	169	408	795	1 295	1 515	1 147	547	105
Ct	M	19 183	16	4	7	21	16	22	33	70	123	200	412	738	1,178	1 989	2 985	4 117	3 944	2 456	862
	F	22 544	4	3	9	13	15	19	44	50	[illegible]	209	432	623	1 081	2 071	3 519	4 974	4 960	3 132	1 284
Primary diagnosis as percentage of total																					
Dp/Dt	M	32 6			100	80	90	86	93	83	73	55	50	43	35	30	28	26	25	30	22
	F	30 5		33	100	80	100	87	91	76	84	51	55	36	37	35	29	28	23	25	25
Cp/Ct	M	70 7	50	75	43	52	38	59	67	77	76	73	75	71	72	69	70	71	70	70	72
	F	71 1	25	33	56	54	40	[illegible]	73	76	75	77	76	74	72	71	71	71	71	70	74
Autopsies per cent																					
Dpa/Dp	M	35 7			100	50	78	53	51	[illegible]	44	45	46	50	39	41	37	[illegible]	22	15	0
	F	32 7		100	100	75	50	69	61	37	62	39	54	48	42	40	30	28	21	15	12
Dca/Dc	M	30 3				0	100	C0	0	50	53	52	55	50	41	36	35	27	22	11	3
	F	27 8		100		100		75	67	67	60	59	42	41	34	36	32	27	20	[illegible]	11
Dta/Dt	M	32 1			100	40	80	54	48	[illegible]	47	[illegible]	50	[illegible]	40	38	36	27	22	12	3
	F	29 3		100	100	80	[illegible]	77	62	44	61	49	49	43	37	37	31	27	21	18	11
Cta/Ct	M	28 9	94	75	100	90	88	86	76	[illegible]	72	[illegible]	63	59	46	41	35	25	18	13	9
	F	26 0	100	100	100	77	[illegible]	[illegible]	77	[illegible]	69	[illegible]	61	50	43	35	32	24	18	13	9
graduated	M+F	27 3	68	80	90	80	80	80	77	74	71	64	62	55	44	38	33	25	18	13	9

Header note (spanning age columns): Number of deaths percentage [illegible]mary diagnos s and percentage autops es in age group (age)

(e) *general underregistration*, irrespective of age, cause, domicile and time (which of course cannot be analysed on the basis of statistics on mortality and causes of death alone)

Registration of DM as the primary cause of death and the frequency of autopsies by sex and age

In Table 23 there are shown—in addition to the total numbers of deaths from DM by sex and age (Dp, Dc Dt)—the registration of DM as the primary cause of death (the quotients Dp/Dt) and the frequency of autopsies (the quotients Dpa/Dp, Dca/Dc Dta/Dt) by sex and age For comparison certain corresponding data are given for cerebrovascular disease viz the number of deaths (Ct) primary diagnosis as a share of the total (Cp/Ct) and the frequency of autopsies (Cta/Ct) On the last line of the table there are calculated graduated frequencies Cta/Ct by age (but with both sexes taken together) to be used for certain standard calculations [1]

By sex and age the frequencies to which DM and cerebrovascular disease are recorded as the primary cause (the quotients Dp Dt and Cp/Ct) are illustrated in Fig 8

The absolute numbers of deaths before age 40 are small and the figures may have been considerably affected by chance fluctuations After age 40 the frequency figures show a marked decrease for DM but a comparatively slight decrease for cerebrovascular disease, there are no apparent differences between males and females A consequence of these trends by age is that for DM a study of primary causes of death will give a very "skew picture, with an underestimation of mortality from DM which gradually becomes more serious with increasing age because of this type of 'skewness' it is evident that statistics based on primary causes of death alone may lead to erroneous conclusions in respect of the age variations of the prevalence of DM [2]

The autopsy frequencies for deaths from DM (total Dta/Dt) and for deaths from cerebrovascular disease (total Cta/Ct) are shown in Fig 9 Under age 70, the autopsy frequencies are lower for DM than for cerebrovascular disease, in the higher age groups the frequency figures are fairly similar There are no apparent differences between males and females The frequencies show a steady decrease with age [3]

Frequency of autopsies by cause

Table 24 shows—in the form of age standardized comparisons for the whole

[1] For the lowest age groups the figures are based on official statistics concerning the frequency of autopsies in Groups XIV (A127–A129) and XV (A130–A135)

[2] From Fig 8 it can be seen that with regard to the relative significance of cerebrovascular disease in different age groups the risks for false conclusions on the basis of the Swedish statistics on primary causes of death are inconsiderable

[3] Since there are great geographical differences in respect of autopsy frequencies—with particularly high figures in the largest cities and very low figures in certain counties (cf p 90) —the variability of these series for the whole country is lower than would have been the case in a homogeneous series On the other hand it should not be overlooked that the age distribution of the population varies between the counties the older age groups are over represented in the rural parts of most counties and underrepresented in the large cities

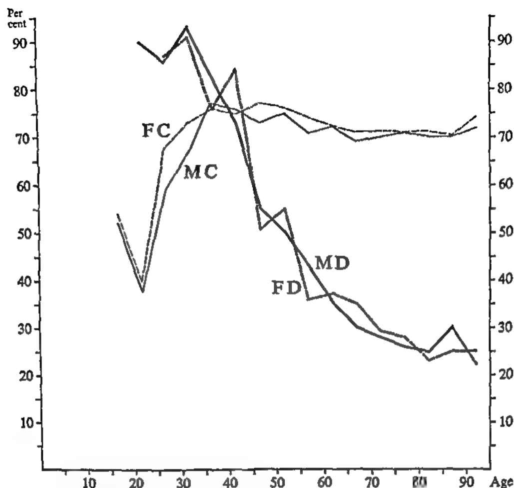


Fig 8 Frequency of primary diagnosis for DM (Dp/Dt) and cerebrovascular disease (Cp/Ct) by sex and age 1961–63

country—the frequency of autopsies for different causes of death (the graduated quotients Cta/Ct by age given in Table 23 being used as standard)

The autopsy frequencies are highest for digestive diseases (69 %) and high also for the group other diseases (55 % about 60 per cent of this group are infant deaths Groups XIV and XV) infective diseases (51 %) genito urinary diseases (47 %) violence (45 %) and neoplasms (41 %) For DM as primary cause the percentage is 34, for DM as primary or contributory cause it is 30 However as can be seen from the age standardized figures, the high autopsy frequencies for ' other diseases violence and neoplasms are in the main explained by the age composition of these deaths After age standardization the autopsy figures for digestive diseases stand out as very high (index 189 with total deaths = 100), also

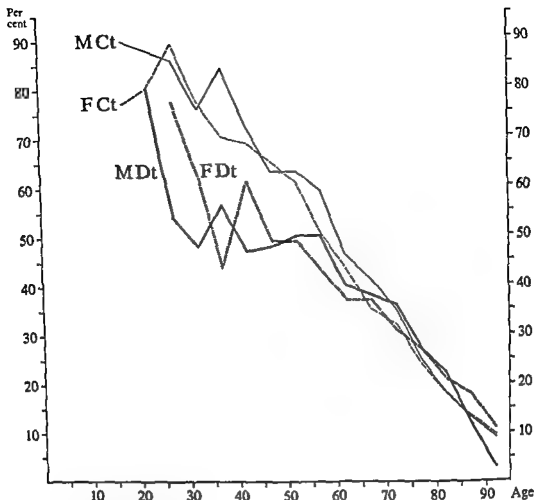


Fig 9 Frequency of autopsies for DM (Dta/Dt) and cerebrovascular disease (Cta/Ct) by sex and age 1961–63

above the average are genito urinary diseases (index 129) infective diseases (109) respiratory diseases (104) and neoplasms (101) For DM as primary or contributory cause the index is 91

Mortality from DM by county and in rural and urban areas

During earlier periods there were great differences in respect of mortality as between the large cities and the country as a whole Successively, these differences have decreased, and nowadays there are no significant differences to be found for the females During the period 1951–60 the death rates for males above 35 were higher in Stockholm than in all Sweden, the excess mortality was about 20 per cent at ages 35–44 and 30 per cent in the higher age groups

For the 4 year period 1959–62 the

Table 24 Age standardized comparison between observed and expected number of autopsies 1961–63

Cause of death		Number of deaths	Autopsies		Raw index	Observed number of autopsies as percentage of expected number	
			Number	Percentage of deaths	(a)	(b)	(c)
Primary cause							
Infect	I	2 132	1 081	50 7	143	117	109
Neopl	II	44 317	18 077	40 8	115	109	101
Diab	D	3 324	1 128	33 9	96	96	90
Nerv	VI	31 927	8 165	25 6	72	90	83
Circ	VII	90 059	26 894	29 9	84	106	99
Resp	VIII	12,832	3 792	29 6	84	112	104
Digest	IX	9 450	6 527	69 1	195	203	189
Gen urin	X	6 393	3 000	46 9	132	139	129
Other dis		8 989	4 957	55 1	156	103	95
Unknown	XVI	2 981	136	4 6	13	25	23
Violence	XVII + XVIII	14 402	6 547	45 5	129	[illegible]	83
Total		226 806	80 304	35 4	*100*	108	*100*
Diabetes							
Dp		3 324	1 128	33 9	96	96	90
Dc		7 273	2,095	28 8	81	100	93
Dt		10 597	3,223	30 4	86	[illegible]	91
Cerebrovascular disease							
Cp		29 600	7 440	25 1	71	[illegible]	85
Cc		12,127	3 964	32,7	92	120	111
Ct		41 727	11 404	27 3	77	*100*	[illegible]

Raw index (a) = Percentages adjusted to give 100 for total deaths
Expected (b) = Calculated with graduated quotients Cta/Ct by age (for the whole country)
(c) = Adjusted to give 100 for total deaths

Central Bureau of Statistics has published special data on mortality by counties giving age standardized mortality ratios by sex and primary cause of death an extract from these statistics is given in Table 25, covering all deaths and deaths from diabetes A63, cerebrovascular disease A70, and arteriosclerotic and degenerative heart diseases A81 [1]

The geographical variations in respect of deaths from DM (*primary cause*) are illustrated in Fig 10 in the form of a profile diagram In view of the results with regard to DM (primary and contributory cause) during the period 1961–63, the "official order of the counties has been modified (G has been moved after K, and Y and Z have been interchanged) The diagram shows the age-standardized mortality ratios for DM, and the quotients between the DM ratios and the corresponding ratios for all causes of death

[1] It should be noticed that 10-year age groups were used this may result in spurious effects but for the present purpose of elucidating certain geographical differences in mortality (primary cause) the data can be regarded as sufficiently accurate

Table 25 Age standardized mortality ratios by sex and county for all causes of death, and the primary causes diabetes (A63), cerebrovascular disease (A70), and arteriosclerotic and degenerative heart diseases (A81) 1959–62

County	Standardized mortality ratio 1959–62 (primary cause)							
	All causes		A63		A70		A81	
	M	F	M	F	M	F	M	F
A	120	96	109	75	84	76	121	99
B	99	100	102	115	90	98	98	100
C	92	97	78	104	73	[illegible]	93	94
D	100	104	113	139	114	116	99	107
E	101	105	113	128	100	98	105	115
F	99	100	129	134	99	108	93	105
G	85	94	98	66	82	97	83	94
H	92	101	113	122	101	112	94	100
I	103	112	129	106	120	113	86	103
K	91	98	58	92	80	87	94	100
L	[illegible]	92	[illegible]	67	90	93	[illegible]	82
M	97	91	62	60	88	82	93	84
N	[illegible]	91	[illegible]	82	96	[illegible]	97	106
[illegible]	101	94	95	90	97	83	106	99
PQ	94	98	120	93	92	96	96	97
R	96	107	120	93	120	122	90	106
S	102	107	112	139	122	125	104	105
T	103	105	105	115	124	120	[illegible]	97
U	98	104	130	117	96	95	96	108
W	103	106	123	122	113	116	107	105
X	104	105	111	124	108	109	105	103
Y	106	110	91	94	127	135	103	106
Z	94	102	82	114	100	122	92	95
AC	105	113	96	133	106	127	115	116
BD	106	112	108	133	119	133	106	116
Total	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Number of deaths								
M	156 306		1 782		18 311		47 468	
F	140 022		2 368		21 901		36 525	

For each sex the ratios show the observed number of deaths as a percentage of the expected number, calculated on the age distribution in the county and the death rates for the whole country (10-year age groups)

From *Mortality by counties 1959–62* Central Bureau of Statistics Official Statistics of Sweden Population and Vital Statistics Stockholm 1964

As can be seen from Table 25 in Stockholm City (A) the male and female [illegible]tios for total deaths are 1 20 and 0 96 However, for DM *both* ratios are considerably lower viz 1 09 and 0 75 A similar situation with lower ratios for DM than for total deaths applies to Gothenburg and Bohus County (O) and Malmöhus County (M), which include the cities of Gothenburg and Malmö and to Blekinge County (K), Kristianstad County (L), Halland County (N) and Västernorrland County (Y) The opposite relation, with higher ratios for DM than

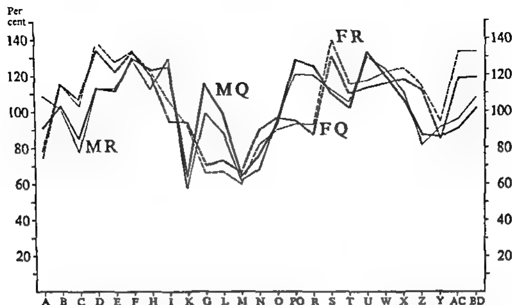


Fig 10 Age standardized mortality ratios (R) by sex and county for DM (primary cause) and quotients (Q) between these and corresponding ratios for all causes 1959–62

for total deaths, is valid in respect of most counties in the middle of Sweden (D, E F H, S, T U, W, X)[1]

In Table 26 for the period 1961–63 an age standardized comparison between the counties (rural and urban areas separated) is performed in respect of deaths from DM It should be stressed that this comparison is based on *death shares* (D/T) and not on death rates (D/P) The table gives the number of deaths (Dp and Dt) by sex and county and further the share of primary cause (Dp/Dt), the quotients of observed and expected number of deaths from DM (O/E for Dp and Dt) and the frequency of autopsies In Table 27 this type of comparison is made for certain large age groups this comparison is restricted to show the rural and urban figures for Sweden and for certain groups of counties, viz Stockholm City (A), Stockholm and Uppsala Counties (B C), the remainder of middle and south-eastern Sweden (a broad belt covering the counties D E F, H I K PQ R, S T, U, W X Z), the south western part (the counties G L, M N, O) and the

[1] According to Marks (1961) in the U S A there exist great regional differences in the certification of causes of death among older persons For instance the highly urbanized states or those with or close to medical centres have low rates for cerebral vascular disease while the predominantly agricultural or rural states record high rates There is an inverse correlation between the number of internists per 100 000 population and the level of death rates from degenerative conditions In New York State the mortality from cerebral vascular disease is relatively, much less in New York City than in the area outside the city Cf also Lew (1957) and Ustvedt (1959)

Table 26 Age standardized comparison between observed and expected number of

County	Number of deaths				100 Dp/Dt		100 O/E				Autopsies per cent			
	M		F		M	F	M		F		M		F	
	Dp	Dt	Dp	Dt			Dp	Dt	Dp	Dt	Dp	Dt	Dp	Dt
All Sweden	1 419	4,359	1 905	6 238	33	31	*100*	*100*	*100*	*100*	36	32	33	29
Rural	768	2 339	1 061	3 410	33	31	104	101	110	107	27	22	25	21
Br	47	113	63	151	42	42	109	[illegible]	121	91	47	32	32	26
Cr	19	57	22	72	33	31	*119*	*110*	*103*	*102*	*68*	*46*	*68*	*43*
Dr	19	67	40	104	28	38	94	103	148	116	38	28	17	16
Er	36	134	61	200	27	31	113	131	139	136	17	22	28	20
Fr	35	127	51	166	28	31	119	137	137	134	14	13	25	19
Gr	31	76	24	81	41	30	118	90	72	71	29	29	33	30
Hr	35	123	68	201	28	34	107	119	155	134	17	13	7	7
Ir	10	36	8	36	28	22	111	128	65	89	10	8	12	19
Kr	16	61	25	89	26	28	94	114	110	116	13	10	12	12
Lr	38	114	45	156	33	29	95	89	85	[illegible]	26	21	24	21
Mr	30	104	37	128	29	29	73	79	69	71	37	29	19	21
Nr	17	51	24	89	33	27	87	81	100	110	18	12	8	13
Or	21	84	29	100	25	29	70	87	77	80	38	26	28	23
PQr	67	167	78	212	40	37	150	117	125	101	40	31	35	29
Rr	41	130	45	174	32	26	118	117	93	108	20	25	13	22
Sr	43	130	73	220	33	33	102	98	136	123	5	10	22	24
Tr	38	[illegible]	41	111	42	37	142	106	114	94	18	22	24	15
Ur	24	70	35	117	34	30	126	120	136	138	42	34	31	33
Wr	39	133	53	231	29	23	90	97	87	116	28	19	21	17
Xr	45	126	60	217	36	28	115	104	115	127	31	21	25	18
Yr	27	96	60	178	28	34	64	72	112	99	26	20	30	27
Zr	22	77	34	117	29	29	87	98	113	120	18	16	15	17
ACr	31	91	47	127	34	37	85	82	112	94	32	31	40	33
BDr	37	82	38	133	45	29	129	97	108	118	22	13	32	18

northernmost part (the counties Y AC, BD) This grouping is based on the data in Table 26

A general feature to be seen from Table 26 is that the variability of the quotients O/E is considerably reduced when the comparison is based on both primary and contributory cause (Dt) and not on the primary cause (Dp) alone However even with the comparison based on Dt certain counties show quotients O/E that are markedly below the average This applies in particular to Stockholm City (0 81 for males and 0 67 for females), and to the counties in the south western part of Sweden (G L, M, N, O rural 0 86 and 0 82, urban 0 89 and 0 88) The urban autopsy percentages are high in the counties comprising the three largest cities (A, O, M) and in the counties B C, PQ and AC With the exception of PQ (Älvsborg County) these counties show comparatively low shares for deaths from DM *In rural Sweden this share*

deaths from DM by sex and domicile (county rural, urban) 1961–63

County	Number of deaths				100 Dp/Dt		100 O/E				Autopsies per cent			
	M		F		M	F	M		F		M		F	
	Dp	Dt	Dp	Dt			Dp	Dt	Dp	Dt	Dp	Dt	Dp	Dt
Urban	651	2 020	844	2,828	32	30	95	99	90	93	45	43	42	39
Au	127	372	141	487	34	29	[illegible]	81	62	67	66	62	65	60
Bu	39	86	47	112	45	42	130	98	112	83	59	51	30	31
Cu	11	39	24	63	28	38	75	88	107	87	45	56	67	51
Du	26	82	43	149	32	29	106	111	126	135	23	34	16	30
Eu	44	151	[illegible]	209	29	30	113	127	118	119	30	32	21	23
Fu	45	115	39	140	39	28	184	154	118	131	18	21	15	19
Gu	–	5	7	21	0	33	0	30	[illegible]	[illegible]		80	57	62
Hu	19	54	17	71	35	24	142	134	90	116	26	20	18	18
Iu	5	9	4	17	56	24	200	114	105	133	20	44	[illegible]	[illegible]
Ku	4	31	13	53	13	25	35	[illegible]	[illegible]	100	25	26	15	4
Lu	7	27	[illegible]	39	26	15	72	90	43	[illegible]	[illegible]	22	67	38
Mu	49	188	65	314	26	21	64	[illegible]	62	92	37	48	55	46
Nu	12	35	18	49	34	37	102	98	115	95	33	17	22	20
Ou	76	240	92	295	32	31	92	99	85	84	75	68	64	65
PQu	28	64	43	97	44	44	115	89	126	89	68	42	54	33
Ru	23	72	15	72	32	21	144	145	66	97	35	28	40	26
Su	23	75	38	99	31	38	116	124	150	119	22	21	16	12
Tu	21	[illegible]	43	118	24	36	84	115	129	109	33	26	35	25
Uu	24	67	29	94	36	31	113	110	105	105	50	39	[illegible]	34
Wu	15	46	22	63	33	35	106	106	113	[illegible]	40	39	27	24
Xu	22	70	30	114	31	26	97	102	101	116	32	31	17	28
Yu	13	45	16	62	29	26	[illegible]	104	83	99	15	33	50	39
Zu	4	14	6	19	29	32	100	114	100	98	0	29	17	21
ACu	7	22	11	28	32	39	99	107	121	98	14	36	73	[illegible]
BDu	7	25	12	43	28	28	63	80	90	104	[illegible]	28	17	16

O = Observed number
E = Expected number calculated with the age distribution of all deaths (by sex and domicile) and the observed shares of deaths from Dp and Dt respectively by sex and age in the whole country

exceeds the average (the index O/E for Dt being 1 01 for males and 1 07 for females) whereas it is below the average in urban Sweden (index 0 99 for males and 0 93 for females) However as can be seen from Tables 26 och 27, this urban deficit is mainly due to the largest cities for the towns in A, O and M the index figures are 0 86 for males and 0 77 for females and for the towns outside A, O and M they are 1 11 and 1 07, respectively, against 1 01 and 1 07 in rural Sweden—in spite of the fact that the frequency of autopsies is considerably higher in these latter towns than in rural Sweden

A closer scrutiny of Tables 26 and 27 reveals a number of interesting details The index figures O/E for Dt are remarkably low for Stockholm City (A) and comparatively low for Stockholm County (B) and urban Uppsala County (Cu), whereas they are high for Sodermanland County (D) Östergötland

Table 27 Age-standardized comparison between observed and expected number of deaths from DM by sex and domicile (certain areas) in groups by age 1961–63

Domicile		Number of deaths All ages	Age 0–49	Age 50–64	Age 65–79	Age 80–	100 O/E for Dt All ages	Age 0–64	Age 65–79	Age 80–	Autopsies Dt per cent All ages	Age 0–64	Age 65–79	Age 80–
Males														
Sweden		4 359	267	721	2 245	1 126	*100*	*100*	*100*	*100*	32	47	33	18
Rural		2 339	131	346	1 173	689	101	105	99	99	22	34	24	11
Urban		2,020	136	375	1,072	437	99	96	101	101	43	59	42	29
A	u	372	24	79	207	62	81	77	[illegible]	76	62	72	62	48
B C	r	170	12	23	87	48	94	92	93	98	36	74	32	17
	u	125	15	23	59	28	95	115	86	97	53	76	53	21
Central belt	r	1 471	85	211	718	457	112	118	108	115	20	[illegible]	23	11
	u	936	65	165	482	224	118	117	116	117	30	48	26	20
South west	r	429	21	64	219	125	86	97	[illegible]	77	24	39	24	14
	u	495	25	91	271	108	89	81	94	88	55	66	56	39
Y, AC BD	r	269	13	48	149	59	[illegible]	80	87	72	22	28	25	7
	u	92	7	17	53	15	98	83	108		33	54	26	20
Females														
Sweden		6 238	173	661	3 605	1 799	*100*	*100*	*100*	*100*	29	44	32	19
Rural		3 410	81	349	1 974	1 006	107	110	108	104	21	36	23	12
Urban		2 828	92	312	1 631	793	93	91	92	95	39	53	40	29
A	u	487	20	44	259	164	67	58	61	85	60	70	60	57
B C	r	223	8	18	130	67	94	79	96	97	32	50	37	15
	u	175	8	23	100	44	85	103	84	76	38	65	42	11
Central belt	r	2 195	50	235	1 265	645	119	126	118	116	19	32	21	11
	u	1 315	34	169	756	356	113	122	111	112	24	39	24	13
South west	r	554	11	48	323	172	82	[illegible]	86	75	21	37	24	11
	u	718	24	61	437	196	[illegible]	74	94	83	52	69	55	39
Y AC BD	r	438	12	48	256	122	103	102	100	109	26	47	27	14
	u	133	6	15	79	33	101	91	101	106	36	57	37	21

O = Observed number

E = Expected number calculated with the age distribution of all deaths (by sex and domicile) and the observed shares of deaths from Dp and Dt respectively by sex and age in the whole country

Central belt = D F F H I K, PQ R S T U W X, Z

South west = G L, M N O

County (E) Jonköping County (F) and Västmanland County (U) the same applies to the towns in Örebro County (Tu) In these latter counties there are large towns and well-equipped hospitals (which are not university clinics) Further, most index figures are comparatively low in the south western part of Sweden but high in Kalmar County (H) which is characterized by a very low frequency of autopsies

In respect of the northern counties a certain time lag in the general decline of mortality should be taken into account Further, as the results are chiefly determined by circumstances obtaining at

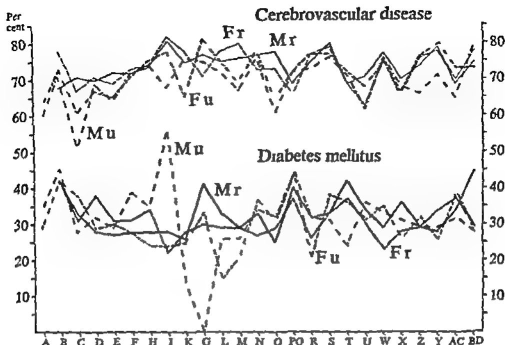


Fig 11 Frequency of primary diagnosis for DM and cerebrovascular disease by sex and domicile 1961–63

higher ages inequalities in the patterns of migration over a long period may influence the data in various ways (selective out migration a skewness in the age composition which is not sufficiently eliminated when 5 year age groups are used, etc)

The geographical variations are illustrated in Figs 11, 12 and 13

By sex and domicile Fig 11 shows—in the form of a profile diagram—the quotients of primary cause for DM (Dp/Dt) and for comparison, the corresponding quotients for cerebrovascular disease (Cp/Ct) In the same way Fig 12 gives the quotients between observed and expected numbers of deaths from DM (O/E for Dt) and the frequency of autopsies (Dta/Dt) It should be noticed that the population of Gotland County (I) is small, and that the population of the urban parts of Kronoberg County (Gu) and Jämtland County (Zu) is small too In the counties A C, M O and AC there are universities with faculties of medicine and university clinics As will appear from Fig 11, the quotients of primary cause for cerebrovascular disease (Cp/Ct) are low in Stockholm City (Au) and in the urban parts of Uppsala County (Cu) Gothenburg and Bohus County (Ou) and Malmöhus County (Mu) The quotients of primary cause for DM stand out as particularly low in urban Malmöhus County (Mu), however taking into account the age composition of the population and the variation of the quotients with age (cf Fig 8)

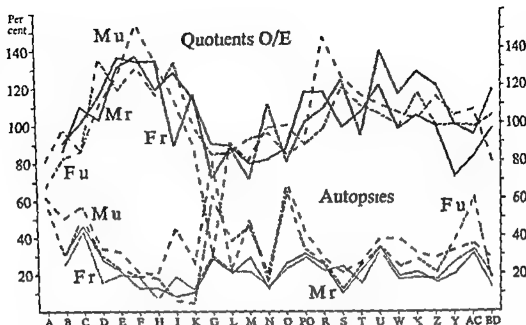


Fig 12 Quotients between observed and expected numbers of deaths from DM, and frequency of autopsies by sex and domicile 1961–63

it is evident that they are low also for Stockholm City (Au) and urban Gothenburg and Bohus County (Ou)

Both for DM and for cerebrovascular disease the variability of the quotients of primary cause appears to be more connected with domicile than with sex and age Although in respect of age the variations are large they seem in the main to follow the same general pattern all over the country The variations in respect of domicile may therefore be assumed to be connected with differences in practice for the "choice" concerning which of two or more causes of death should be stated as the primary cause For instance it may be observed that in Älvsborg County (PQ) the comparatively high quotients for DM are accompanied by comparatively low quotients for cerebrovascular disease in respect of deceased persons with both these diseases there may have been a preference for stating DM as the primary cause

In Fig 13 the correlation between the observed and expected absolute numbers is shown for DM with relative figures the differences in population size between the counties may be overlooked As will be seen from Fig 13, the "deficits" are particularly large for females in Stockholm City (Au), but they are large also for males in Stockholm City and for certain other groups (for instance males in Mu and Yr, females in Ou, Mr and Mu) On the evaluation of the diagram the random error of the observed number (of a magnitude approximately equal to the square root of the expected number) should be taken into account If it is assumed that there is in fact geographical homogeneity in respect of mortality from DM and, further that the gaps are solely due to "underregistration" in cer-

Females

Males

Obs − Exp

+100

0

−100

−200

Obs

100

200

300

400

500

	rural	urban
A	▲	●
B C	▲	▲
Central	◇	◆
South west	▫	■
Y AC BD	▼	▼

Fig 13 Difference between observed and expected numbers of deaths from DM by sex and domicile 1961–63

tain counties (mainly Stockholm City and the counties in the south western part of Sweden) the expected numbers would increase by about 15 per cent for males and about 20 per cent for females, as is indicated by the thick lines in the diagram In addition to these *domicile gaps* there may of course be other types of underregistration all over the country, for instance in respect of sudden death and possibly, in respect of the most advanced age groups (cf Figs 6 and 7), these questions will be discussed in a later connection

Very likely, there is a real gap in the registration of deaths from DM in so far as in the three largest cities interest has been focused on certain other causes of deaths (for instance neoplasms and diseases of the circulatory and digestive systems) the personal contacts between the certifying doctor and the deceased patient and his family may to a greater extent have been comparatively weak (or even absent) and possibly the certificates will sometimes give particular stress to the patho-anatomical findings The alternative explanation that mortality from DM really is lower in the three largest cities than in most other towns in Sweden seems highly improbable [1]

It should be remembered that the majority of the inhabitants in the higher age groups in the three largest cities were not born in the cities but have moved there generally in adolescence or at their younger working ages Therefore the genetic composition of the population of Stockholm, Gothenburg and Malmo cannot deviate considerably from that of the general Swedish population (at least in so far as such a common disease as DM is concerned)

In a way, the most interesting differences in Tables 26 and 27 are between the south western part of Sweden and the 'central belt', already described The rural indices O/E for Dt are 0 86 and 1 12 for males and 0 82 and 1 19 for females, the urban indices (of which the first mentioned ones include the cities of Gothenburg and Malmo) are 0 89 and 1 18 for males, and 0 88 and 1 13 for females Hence, the quotients between the south western' and the central belt' indices will be rural 0 77 for males and 0 69 for females urban 0 75 for males and 0 78 for females, or on average 0 75 It is interesting to notice here that these quotients are fairly similar for both sexes

As may be seen from Table 27 and is further elucidated by the following table, the quotients are also fairly similar for different age groups

Sex	Area	All ages	Age 0–64	65–79	80–
O/E for Dt					
South western part					
M	r	0 86	0 97	0 88	0 77
	u	0 83	0 81	0 94	0 88
F	r	0 82	0 81	0 86	0 75
	u	0 88	0 74	0 94	0 83
Central belt					
M	r	1 12	1 18	1 08	1 15
	u	1 18	1 17	1 16	1 17
F	r	1 19	1.26	1 18	1 16
	u	1 13	1.22	1 11	1 12
Quotient south western part to central belt					
M	r	0 77	0 82	0 81	0 67
	u	0 75	0 69	0 81	0 75
F	r	0 69	0 64	0 73	0 65
	u	0 78	0 61	0 85	0 74

[1] It might be noted that the same situation was established in respect of cerebrovascular disease with low quotients O/E for Ct in Au Ou and Mu (and also in Cu)

On the basis of the statistical data the possibility cannot be excluded that there exists a real difference in so far as the prevalence of DM is lower in the south-western part of Sweden than in the rest of the country—possibly 15 or 20 per cent lower However, in view of the homogeneity of the Swedish population the more likely explanation seems to be that the diagnosing of DM or its reporting in the death certificates (or both) has been more complete in the central belt than in south western Sweden (and in Stockholm City)

To sum up with a certain reservation regarding the south-western part of the country, it seems warrantable to conclude that in Sweden there does not exist any real geographical difference in respect of mortality—and morbidity—from DM (apart from the time lag already mentioned) and further, that the statistical records—after due correction for the gaps connected with unknown cause —contain a certain underestimation of mortality from DM (primary or contributory cause) This ' geographical underestimation may be assessed at about 15 per cent of the number of registered deaths from DM (primary or contributory cause) for males and about 20 per cent for females [1]

Primary cause for deaths with DM as a contributory cause

Table 28 shows the distribution by primary cause of deaths with DM as a contributory cause In all, among deaths other than those for which DM was registered as the primary cause, 3 3 per cent were reported to have had DM as a contributory cause (males 2 5 per cent females 4 1 per cent) Particularly high percentages are found for diseases of the circulatory system (3 4 and 5 7) respiratory diseases (3 5 and 5 3), and diseases of the nervous system and sense organs (3 3 and 4 9), in addition, for females the percentage is high for diseases of the genito urinary system (2 7 and 5 7) On the other hand, the figures for neoplasms are very low (1 0 and 1 4) Among these deaths with DM as a contributory cause the frequency of autopsies stands out as low for the primary causes infective diseases and diseases of the genito-urinary system (cf Table 24) in comparison with the figures concerning cerebrovascular disease as a contributory cause, the frequency of autopsies also stands out as low for neoplasms as the primary cause

However, the distribution by age is not the same for deaths from different primary causes, and therefore some sort of age standardization is warranted This is made on the basis of the idea (in general quite unrealistic) that the registered primary cause is not a cause of death but solely a way of classifying deaths [2] The index figures in Table 28 give the quotient between the observed number of deaths from DM (as a contrib-

[1] It is quite obvious that the statistics on primary cause of death give a considerable underestimation of the occurrence of DM in the three largest cities. The same applies to cerebrovascular disease in respect of Au Ou and Mu (and also Cu)

[2] If blue eyes were without significance for mortality but blue eyes was always recorded as the primary cause of the death and the real (underlying) cause was always recorded as a contributory cause then the distribution by contributory cause in this group would agree with the distribution by cause of all deaths (other than "blue eyes")

utory cause) and the number that would have been registered if the shares of deaths from DM (Dt/T) by sex and age had been applicable irrespective of the primary cause of death [1] The age-standardized index must be low, if the primary cause is a real cause of death which is not (or is only slightly) correlated with DM It may be high in either of two cases, viz. if the registered primary cause is not a real cause of death and if there is a correlation between the registered primary cause and DM As will be seen from the table, the index figures are very low for violent deaths among males and for neoplasms, and low also for the remainder group ' other diseases ', for diseases of the digestive system and for violent deaths among females They are high for circulatory and respiratory diseases and in respect of females, for diseases of the genito urinary system In the main, these results are in agreement with current views concerning the connections between DM and diseases of the circulatory system and the results obtained in previous studies concerning the importance for mortality statistics of variations in the incidence of respiratory diseases in particular influenza epidemics (cf Larsson 1965) In the evaluation of the data in Table 28 due regard must be paid to the heterogeneity of the reports on causes of death (the underestimation of the occurrence of deaths from DM in Stockholm City and the probable underestimation of this occurrence in the south-western part of Sweden)

In the parallel study of mortality from cerebrovascular disease a similar analysis was performed with regard to the distribution by primary cause of deaths with cerebrovascular disease as a contributory cause For comparison, the corresponding autopsy percentages and index figures are stated in Table 28 As will be seen from the table, the index figures are exceptionally high for the primary cause DM and very low for neo plasms [2]

An interesting approach to the study of the prevalence of DM, and the mortality and causes of death among diabetic patients has been made in Denmark by Dreyer and Hey (1953, 1954) Their evaluation is based on a register of DM patients who during the period 1944–48 received special rationing cards for saccarine The prevalence rate was found to be 0 43 per cent (males 0 38 females 0 47), a higher prevalence was registered in the towns (0 52 per cent) than in the rural areas (0 34 per cent) At ages 65–69 the prevalence figures in the towns were 2 06 per cent for males and 2 34 per cent for females in the rural areas they were 0 95 and 1 87, respectively

For 15 400 diabetics living at the end of 1948 there occurred in the period January 1, 1949–June 30 1951 (hence within 2 5 years) a total of 1 539 deaths (670 males 869 females) According to the rules then followed for the classification of causes of death DM should always be given as the primary cause unless cancer tuberculosis or violence was mentioned in the certificate The authors performed a correction (re classification) in so far as all deaths from DM, where a secondary cause was stated were attributed to this cause Further a calculation was undertaken of the expected number of deaths in the relevant period

[1] This method of calculation implies a certain bias in so far as the occurrence of diseases other than DM as a contributory cause is not taken into account but for the purpose of age standardization it is quite sufficient

[2] In one sense the figures for diabetes are especially interesting they give an impression that—weighed with the age distribution of deaths from cerebrovascular disease—diabetes does not cause any appreciable increase of mortality

Table 28 Distribution by primary cause of deaths with DM as a contributory cause 1961–63 For comparison certain corresponding data are given also for deaths with cerebrovascular disease as a contributory cause

Primary cause		Total number of deaths T	Deaths from DM by age: Number Dc Dp All ages	0–34	35–49	50–64	65–79	80	Percentage of deaths D/T	Autopsies for deaths from DM: Number Da	Percentage Da/D	Index for DM by age per cent: All ages	0–49	50–64	65–79	[illegible]	Deaths from cerebrovascular disease: Autopsies per cent Ca/C	Index per cent
Males																		
Infect	I	1 335	38	1	5	12	18	[illegible]	2 8	15	39	[illegible]		100	83		47	19
Neopl	II	23 0[illegible]	235	1	4	44	126	60	1 0	[illegible]	37	27	10	24	26	38	52	12
Nerv	VI	14 769	486	1	6	51	288	140	3 3	118	24	85	44	[illegible]	92	79	26	
Circ	VII	[illegible] 598	1 661	–	28	257	920	456	3 4	473	28	90	71	89	[illegible]	87	32	39
Resp	VIII	6 511	225	1	1	16	113	94	3 5	60	27	95		74	99	[illegible]	26	62
Digest	IX	4 884	111	1	[illegible]	23	57	[illegible]	2 3	76	68	63	25	66	61	73	66	22
Gen urin	X	4 035	108	1	2	12	59	34	2 7	34	31	71		65	78	70	31	37
Other dis		4 739	32	–	3	8	17	4	0 7	13	41	46		47	53	34	58	31
Unknown	XVI	1 277	–	–	–	–	–	–	–	–		–			–	–		
Violence		9 836	44	4	1	8	[illegible]	13	0 4	17	39	15	5	11	23	44	42	14
Subtotal		119 083	2 940	10	52	431	1 616	[illegible]	2 5	892	30	68	24	60	73	74	35*	32*
Diab	Dp = A63	1 419	1 419	87	118	290	629	295	100 0	506	36	(259)					36	96
Total		120 462	4 359	97	170	721	2 245	1,126	3 6	1,398	32	*100*	*100*	*100*	*100*	*100*	26	*100*
Females																		
Infect	I	797	34	[illegible]	2	3	15	12	4 3	10	29	79			59		36	18
Neopl	II	21 258	293	–	6	45	187	55	1 4	95	32	24	10	[illegible]	[illegible]	29	54	10
Nerv	VI	17 1[illegible]	838	[illegible]	2	66	515	253	4 9	173	21	78	27	[illegible]	79	78	24	
Circ	VII	41 461	2 373	1	9	221	1 344	[illegible]	5 7	619	[illegible]	93	57	122	[illegible]	92	29	43
Resp	VIII	6 321	336	2	–	16	195	123	5 3	[illegible]	25	92		94	100	83	18	49
Digest	IX	4 566	174	1	2	16	110	45	3 8	95	55	63		53	66	62	51	17
Gen urin	X	2 358	135	–	5	25	[illegible]	[illegible]	5 7	55	41	100		106	105	95	41	38
Other dis		4 250	34	[illegible]	2	8	15	7	0 8	19	56	27	35	39	21	29	47	22
Unknown	XVI	1 704	–	–	–	–	–	–	–	–		–			–	–		
Violence		4 566	116	–	–	7	54	55	2 5	54	47	53	0	25	59	[illegible]	38	29
Subtotal		104 439	4 333	10	[illegible]	407	2 515	1 373	4 1	1 203	[illegible]	71	23	[illegible]	72	77	31*	33*
Diab	Dp = A63	1 905	1 905	72	63	254	1 090	426	100 0	622	33	(149)					25	77
Total		106 344	6 238	[illegible]	91	661	3 605	1,799	5 9	1,825	29	*100*	*100*	*100*	*100*	*100*	24	*100*

Index = Quotient between observed number of deaths from DM and the number that would have been registered if the shares for death from DM (Dt/T) by sex and age had been applicable irrespective of the primary cause of death (and corresponding calculation for deaths from cerebrovascular disease)

* In the two columns to the right the subtotals refer to deaths from all primary causes except Cp

on the assumption that the mortality (by sex, age and primary cause of death) was the same as in the population as a whole The overall mortality ratio (the quotient between observed and expected number of deaths) was found to be 670 368 = 1 82 for males and 869/501 = 1 73 for females The mortality ratio for cancer was 62/69 = 0 90 for males and 87/100 = 0 87 for females Of all deaths 9 7 per cent were classified as due to cancer In a study of death certificates in which DM is mentioned Heintzelmann (1952) found no more than 4 8 per cent of deaths from cancer Dreyer and Hey consider it likely that the occurrence of DM will often be neglected where cancer is the primary cause of death

With regard to contributory causes there are no reasons for assuming that the records exaggerate the occurrence of DM On the other hand where the records are based on notification or where the certifying doctor has inspected the body but has not seen the patient before death there may occur a certain overdiagnosing in respect of deaths from the primary cause cerebrovascular disease especially because sudden death from diseases of the circulatory system may have erroneously been interpreted as death from cerebrovascular disease However the quantitative effect of this overdiagnosing must be comparatively slight (cf Larsson 1965, 1967)

The statistics for different primary causes of death on the occurrence of DM as a contributory cause give a clear impression that there is an underregistration of DM for at least deaths from the primary causes neoplasms (II) and violence ((accidents and suicide XVII - XVIII) The indices given in Table 28 for all primary causes except DM are 0 68 for males and 0 71 for females, in respect of neoplasms the figures are 0 27 and 0 24, and for violence they are 0 15 and 0 53, respectively Further, there seems to be an underregistration for the remainder group 'other diseases with indices 0 46 and 0 27, and possibly also a slight underregistration for digestive diseases with indices 0 63 for both sexes Although there may be an association (or even a causal connection) between DM and the quantitatively dominating groups circulatory diseases (indices 0 90 and 0 93) and diseases of the nervous system and sense organs (indices 0 85 and 0 78) it does not seem likely that there really is a strong negative connection between DM and the first mentioned categories of causes of death implying for instance that the presence of DM would give a certain degree of protection against neoplasms or that neoplasms would give a protection against DM, nor does it seem likely that DM protects against accidents and suicide to a marked degree—although psychological arguments may be adduced in favour of a theory that certain effects of this kind exist (in addition, it should be observed that insulin has anti-depressive effects)

For comparison certain results from the comprehensive study by Westlund (1966) may be mentioned Westlund investigated diabetic patients resident in Oslo Norway who had been registered for the first time in thirteen hospital departments in Oslo during the period 1925-54 The patient file was matched with lists of deaths in Oslo and Aker for 1937-39 and 1941-55 patients discharged alive were subjected to a mortality follow up (until 1961) The completeness of the death certificates for 2,664 deceased diabetic patients can be seen from the following table (after Westlund s Table V)

Primary cause of death	Number of deaths	DM mentioned per cent
Tuberculosis	93	72
Tumour	247	54
DM	311	96
Apoplexy	472	68
Coronary	545	63
Resp	209	71
Digest	96	45
Gen urin	127	74
Suicide	13	31
Accident	57	53
Other or unknown	494	64
Total	2 664	67

* Underlying cause of death as revised on basis of all available information

A highly interesting feature is revealed from the variation of the index figures with age In Table 28 a division has been made into four age groups viz 0–49 50–64 65–79 and 80 and over

In respect of violence the total index is 0 15 for males and 0 53 for females, for the age group 0–49, the indices are 0 05 for males (5 deaths with DM as a contributory cause against 112 expected) and 0 for females (36 deaths with DM as a contributory cause expected but none registered) at ages 80 and over the index figures for violence are 0 44 for males and 0 87 for females Here it should be remembered that deaths from accidents in particular at younger ages often occur outside the town or district where the deceased person was domiciled

For both sexes there is a marked rise with age in the index figures not only for *deaths from violence but also for deaths* from neoplasms According to the rules for the classification of causes of deaths violence should as a rule be stated as the primary cause According to earlier principles the same should apply in respect of cancer (except in the event of coincidence with death from violence) and therefore it seems likely that the low index figures for neoplasms should be interpreted as mainly due to a real underregistration of DM [1]

Taking into account the geographical distribution of deaths from different primary causes, it can be estimated that the underregistration of DM as a contributory cause in the case of certain groups of primary causes of death—the *cause gaps*—amounts to about 10 per cent for males and about 8 per cent for females (in addition to the domicile gaps of 15 and 20 per cent, respectively) for both sexes a gap of 7 per cent relates to neoplasms as the primary cause, whereas 3 per cent for males and less than 0 5 per cent for females relate to violent deaths In this assessment, account has been taken of the geographical variations of the registered mortality from neoplasms and violence (primary cause)

Registered mortality from DM before 1961

It is often said that the prevalence of DM (in a certain group by sex and age) has been increasing with time which would imply that mortality from DM has been increasing with time [2] With regard

[1] As can be seen from Table 28 the index figures in respect of deaths with cerebrovascular disease as a contributory cause are very low for the primary causes neoplasms and violence In fact these experiences support the theory that there is a real underregistration of contributory causes for the primary causes neoplasms and violence

[2] For some groups by sex and age the rising prevalence might have been counterbalanced by reduced excess mortality but in respect of the death shares this cannot apply to all age groups at higher ages the excess mortality for diabetics must have been fairly moderate

to Sweden available statistics on primary (main) causes of death give a clear impression of a rising mortality from DM at ages above 70, for ages below 70 the picture is more complicated However, since the statistics do not contain any information on *contributory causes of death* it has been almost impossible to arrive at conclusive data

In Table 29 a survey is given for 10-year periods 1911-60 and the 3 year period 1961-63 The data are based on published official statistics concerning deaths from DM (main or primary cause) total deaths and population The average annual number of deaths from DM and the death shares and death rates for DM are given by sex in 5- or 10-year groups by age ages above 70 are taken together (for the period before 1948 statistics by cause of death with a finer age division are not available) It should be noticed that the classification rules were altered from 1931 and again as already mentioned from 1951 (and in some respects from 1960)

The decrease in the death rates from 1921-30 to 1931-40 (for ages below 70 or 60) may in part be connected with altered rules for selecting which of two or more diseases should be stated as the main cause of death (hence in fact a certain transference of DM from main cause to contributory cause) The decrease from 1931-40 to 1941-50 (like the rise of the death rates thereafter) has been ascribed to the rationing of food during the Second World War Whether this is the real explanation (or part of it) cannot however be decided on the basis of statistics on primary causes of death alone

As already mentioned Westlund (1966) has performed a comprehensive study of the incidence of DM in Oslo He summarizes his results as follows

For ages below 30 the incidence has remained practically constant throughout the period 1925-54 There is an incidence maximum at age 15-19 in both sexes At older ages there is a pronounced drop in incidence during the 1940-45 war and a return to pre war levels is completed by 1946 The pre war level is not exceeded however except in age groups 70 and over The post war rise in incidence is probably due to more efficient case finding

It has already been mentioned that in 1961-63 the death shares are high for the younger adult ages As can be seen from Table 29 this situation did not exist in 1931-40 it has been brought about through the marked decline in total mortality in these age groups shown in Table 20 (cf Larsson 1965) Further, it is interesting to notice that at ages 5-14 the death shares have gone down markedly in spite of the general decrease of mortality at these ages

The trend in the death rates does not indicate a real increase of mortality from DM the observed increase at ages above 70 can be wholly explained from successively more complete registration If so it may be warrantable to conclude that there has been a successively more complete registration for deaths at working ages also and that this has been largely counterbalanced by a reduction of the excess mortality for diabetics

The developments after the introduction of the W H O code in 1951 are elucidated in Table 30 in which the number of deaths from DM (primary cause) is shown over the years 1951-63 Using the death shares (Dp/T) by sex and age

Table 29 Mortality from DM (main cause, primary cause) by sex and age 1911–63

Sex Attained age	Average annual number of deaths from DM						Death share DM deaths per thousand deaths						Death rate DM deaths per 100 000 population					
	1911–20	1921–30	1931–40	1941–50	1951–60	1961–63	1911–20	1921–30	1931–40	1941–50	1951–60	1961–63	1911–20	1921–30	1931–40	1941–50	1951–60	1961–63
Males																		
0– 4	10	10	5	3	1	–	1	2	2	1	1	–	3	4	2	1	0	0
5– 9	16	15	7	2	0	–	15	25	18	10	2	–	5	5	3	1	0	0
10–14	20	17	6	2	2	1	25	34	17	11	11	9	7	6	2	1	1	0
15–19	25	18	7	2	2	1	18	19	11	7	8	5	9	6	3	1	1	0
20–29	32	23	7	6	16	15	9	10	4	5	27	29	7	5	1	1	3	3
30–39	32	23	10	7	22	25	12	12	6	5	24	34	9	6	2	1	4	5
40–49	28	22	13	9	21	27	12	10	6	4	12	16	10	7	3	2	4	5
50–59	46	42	36	21	34	57	14	14	10	6	9	13	20	17	12	6	8	11
60–69	62	83	78	48	60	100	12	16	13	7	10	12	36	42	36	18	22	28
70–	60	90	118	92	164	247	5	6	7	5	8	11	45	61	71	50	71	94
Total	331	343	287	192	331	473	8	10	8	5	9	12	12	12	9	6	9	13
Females																		
0– 4	8	9	4	1	1	–	1	2	2	1	1	–	3	3	2	0	0	0
5– 9	16	15	6	3	1	1	16	29	22	17	12	7	6	6	3	1	0	0
10–14	23	20	10	4	2	0	27	38	33	34	26	4	8	7	4	2	1	0
15–19	19	16	8	4	2	1	14	18	12	15	15	11	7	6	3	2	1	0
20–29	28	21	10	9	16	12	10	10	6	10	55	52	6	4	2	2	3	3
30–39	23	17	10	7	19	16	9	9	6	6	31	38	6	4	2	1	4	3
40–49	25	24	14	11	14	15	11	11	6	6	10	13	8	7	3	2	3	3
50–59	43	48	48	30	37	38	14	16	15	10	13	14	16	17	15	8	8	8
60–69	65	112	133	92	125	149	13	20	23	16	21	26	32	48	55	32	36	38
70–	58	122	206	164	271	403	4	7	11	8	12	17	33	65	99	73	99	125
Total	308	404	449	325	488	635	7	11	12	9	14	18	11	13	14	10	13	17

* Main (primary) cause of death 1911–30 code 47 1931–50 code 2360 1951–63 WHO code 260 = A63

Table 30 Age standardized comparison between observed and expected mortality from DM (primary cause) 1951–63

Year	Annual number of deaths from DM (primary cause)						100 O E for Dp					
	Males			Females			Males			Females		
	Rural	Urban	All	Rural	Urban	All	Rural	Urban	All	Rural	Urban	All
1951	162	115	277	268	195	463	66	65	66	76	71	74
1952	204	138	342	299	212	511	86	75	81	90	77	84
1953	151	128	279	266	239	505	63	67	65	78	84	81
1954	149	126	275	263	202	465	63	67	65	79	72	76
1955	159	148	307	233	181	414	68	77	72	73	64	69
1956	172	144	316	239	187	426	72	71	72	75	64	70
1957	195	156	351	250	234	484	79	75	77	75	79	77
1958	180	149	329	265	216	481	77	73	75	82	73	78
1959	229	169	398	284	260	544	97	83	91	90	88	89
1960	233	207	440	293	293	586	94	93	94	89	94	91
1951–55	165	131	296	266	206	472	69	70	70	79	74	77
1956–60	202	165	367	266	238	504	84	79	82	82	80	81
1961–63	*256*	*217*	*473*	*354*	*281*	*635*						
1951–55	Corrected for deaths from unknown cause (XVI)						74	71	73	85	75	80
1956–60							86	80	83	85	81	83
1961–63							104	95	*100*	110	90	*100*

1961–63 as standard and multiplying these shares by the total numbers of deaths by sex and age in each of the years 1951–60 the expected numbers of deaths from DM (primary cause) have been calculated the quotients between the observed and the expected numbers are given in the table for all Sweden and for rural and urban areas [1]

As will be seen from the table there is a distinct increase in the index figures During the period 1951–55 the figures—corrected by proportionate distribution of deaths from unknown cause (XVI) over known causes (within each group by sex and age)—were on average 0 73 for males and 0 80 for females during the period 1956–60 they had risen to 0 83 and 0 83 respectively whereas during the period 1961–63 they are by definition 1 00 The urban figures are lower than the rural ones (0 71 and 0 75 against 0 74 and 0 85 in 1951–55, 0 80 and 0 81 against 0 86 and 0 85 in 1956–60, 0 95 and 0 90 against 1 04 and 1 10 in 1961–63)

These great changes cannot reasonably be explained as being due to a real increase in the prevalence of DM (by sex and age) but must be ascribed to a successively more complete registration of DM in part connected with an augmented interest in the diagnosing of the disease and possibly to a successively growing inclination among certifying doctors to state DM as the primary cause of death (and not as a contributory cause)

For comparison certain statistics from the U S A *may be mentioned*

[1] For the separate years 1951–60 no correction was made for deaths from unknown cause (XVI) For the 5 year periods 1951–55 and 1956–60 there are also given corrected values (deaths from unknown cause being proportionately distributed over known causes within each group by sex and age)

Moriyama (1964) in his thorough analysis of death rates by cause over the period 1930–60 emphasizes that the diabetic mortality trend is difficult to assess because of the major changes in the disease classification procedures in 1949 For white males and females aged 45–54 the registered death rates for DM fell from about 0 18 and 0 30 per thousand in 1930–39 to less than 0 10 around 1955 A similar pattern was seen in the age group 55–64 with a decrease from about 0 6 to 0 3 per thousand for males and from about 1 1 to 0 4 per thousand for females

Klebba (1966) studying mortality trends in 1954–63 reports an increase of the general death rate for DM from 0 156 per thousand in 1954 to 0 172 per thousand in 1963 or by 10 per cent For white males the increase was about 12 per cent (from 0 127 to 0 142) there was comparatively little change for white females (increase from 0 188 to 0 192) For non white males and females the relative increase over the 10-year period was marked viz over 40 per cent in both sexes (from 0 098 to 0 139 for males and from 0 181 to 0 254 for females) The changes for certain age groups can be seen in the following table

Year	Death rate for DM (underlying cause) per 100 000 population in groups by age and sex							
	45 54		55 64		65 74		75 84	
	M	F	M	F	M	F	M	F
1954	10	12	29	47	70	112	131	172
1955	10	12	30	44	71	111	131	166
1956	10	12	31	44	71	111	130	169
1957	10	12	29	46	72	112	128	167
1958	11	12	30	44	73	108	129	170
1959	11	12	31	44	72	106	134	171
1960	12	12	32	44	76	108	145	178
1961	11	11	31	40	75	106	139	181
1962	12	12	33	41	79	107	141	175
1963	12	12	33	41	80	107	145	182

United States After Klebba (1966)

The developments in the different counties are elucidated in Table 31 The table states for the years 1955–64, the share of deaths from DM (primary cause), both sexes are taken together, and no age standardization is made At the bottom of the table are given figures for groups of counties In addition the total numbers of deaths in 1955 and 1964 are given

As can be seen from the table, the series for Stockholm City (A) does not show any increase with time the same applies to Uppsala County (C) and Malmohus County (M) For the south-western counties (G L M N O) taken together, there is found a certain increase from 9 per thousand in 1955–57 to about 12 per thousand in 1961–64 For the northernmost counties (Y, AC, BD) the shares have risen from about 9 per thousand in 1955–57 to about 15 per thousand in 1961–64, and for the central belt there has occurred a gradual rise from 12 to about 18 per thousand For total deaths from DM (Dt) in 1961–63 too the death shares are lower in Stockholm City and in the south western counties than in the central belt although the relative differences are less marked than in respect of primary cause alone It is very likely therefore, that there has occurred—except for Stockholm City—a certain transfer from the classification of DM as a contributory cause to its classification as the primary cause of death It must be regarded as quite out of the question that the series in Table 31 are an expression of a real increase in the prevalence of DM and they must not be taken as evidence for the existence of real differences in respect of the prevalence of DM in different parts of the country

Table 31 Death shares for DM (primary cause) by county 1955–64

County	Number of deaths thousands		Deaths from DM (primary cause) per thousand deaths									
	1955	1964	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964
A	7 2	8 7	10	9	14	11	14	13	10	12	10	10
B	3 3	3 9	10	11	11	12	16	12	15	18	19	17
C	1 6	1 7	16	11	14	13	9	13	17	14	14	11
D	2.1	2 5	10	11	8	10	16	18	21	15	17	20
E	3 6	3 9	11	15	14	15	17	17	18	14	21	19
F	2 7	2 8	11	14	12	15	18	20	17	21	22	18
G	1 5	1 7	19	14	15	8	13	8	18	9	10	12
H	2 5	2 6	14	10	8	16	16	15	21	14	20	25
I	0 7	0 7	13	6	20	16	6	16	16	17	9	18
K	1 4	1 7	13	9	17	8	12	11	8	13	15	8
L	2 4	2 8	7	10	7	6	11	11	12	11	12	16
M	5 6	6 5	9	10	10	10	9	9	10	8	10	12
N	1 7	1 7	9	9	9	13	12	7	14	13	15	16
O	5 1	6 1	7	7	8	7	12	14	13	14	11	14
PQ	3 5	3 8	14	14	12	15	12	13	17	18	21	17
R	2 6	2 7	11	16	18	12	12	15	16	13	15	16
S	3 1	3 3	13	10	11	11	15	17	19	15	21	18
T	2 6	2 6	11	10	10	14	12	15	19	14	19	24
U	2 0	2 1	8	14	14	14	18	16	14	20	20	21
W	2 8	3 1	9	14	10	13	19	16	14	16	12	19
X	2 9	3 2	13	10	12	14	14	13	18	17	13	16
Y	2 8	3 0	6	5	11	9	8	12	15	13	12	16
Z	1 3	1 5	10	9	12	13	17	12	13	13	18	17
AC	2 0	2 1	5	6	8	6	9	14	17	18	11	15
BD	1 8	2 0	13	9	9	11	15	18	14	15	17	19
Rural	37 9	38 9	9	11	11	12	14	13	16	15	16	
Urban	30 7	37 8	11	10	12	11	13	14	14	14	14	
Total	68 6	76 7	11	11	11	11	13	14	15	14	15	16
A	7 2	8 7	10	9	14	11	14	13	10	12	10	10
B C	4 9	5 6	12	11	12	12	14	13	16	17	17	15
Central belt	33 7	36 5	12	12	12	13	15	15	17	16	18	19
South west	16 2	18 8	9	9	9	8	11	11	12	11	11	14
Y AC BD	6 6	7 0	8	7	10	9	11	14	15	15	13	16

Obviously the question of a possible *time gap* in the statistics on deaths from DM (primary *and* contributory cause) during the period 1961–63 cannot be answered through the available data on mortality by primary cause On the other hand it should be emphasized that there is nothing in the statistics to support a theory that there has been an increase of the prevalence of DM (in a certain group by sex and age) apart from the effects arising from the reduction of the excess mortality among diabetics

As mentioned already, for the period 1961–63 the quotient Dp/Dt is less than one third, furthermore, this quotient shows great variation with age, and there are considerable differences by domicile It cannot be decided with certainty whether or not the 'skewness of the quotient has changed with time since only the numerator and not the denominator in the quotient is known From Tables 29–31 it can be concluded that the registration of DM as the primary cause of death must have been markedly

less complete in earlier periods than in 1961–63 Although it cannot always be regarded as incorrect to draw conclusions from such biased samples as the data on deaths from the primary cause DM represent it must, from a purely methodological standpoint be a necessary condition that the nature and magnitude of the existing biases can be properly evaluated[1] Estimates, sometimes quite good ones have been made about the representativity of the data on primary causes of death—one may say in the form of guesses based on general knowledge—but as far as we know a study of the problems on the basis of quantitative *observations* has not been carried out previously

Mortality from DM in 1965

Recently the Central Bureau of Statistics published its report on causes of death in 1965 For the first time data on contributory causes (and complications) have been included for the whole country and with division by sex (but not with division by age) the recorded statements on causes of death (primary cause a maximum of 3 complications and a maximum of 4 contributory causes) are presented according to the Detailed List On the basis of these statistics Table 31A has been prepared by groups of causes it shows the total number of statements on causes of death and the distribution by type (p = primary cause, c = complication c = contributory cause) As will be seen from the table DM as the primary cause (Dp) covers 31 per cent of all statements regarding DM (Dt) The percentages are particularly high for suicide (males 89 females 97) neoplasms (89, 91) and, in respect of males, for accidents (83 54)

[1] Seen against the statistics presented in Tables 21–28 the data in Tables 29–31 may in part serve as a warning against the use of certain types of biased samples

Table 31A Deaths by sex and multiple causes 1965

Cause of death		Number of statements								p as percentage of total	
		Total		p		c		c			
		M	F	M	F	M	F	M	F	M	F
Infect	I	765	547	389	251	87	91	289	205	51	46
Neopl	II	8 895	7 911	7 908	7,200	–	–	987	711	89	91
DM	D	1 810	2 490	565	769	–	–	1 245	1 721	31	31
Nerv	VI	7 322	8 459	4 598	5 462	745	1 007	1 979	1 990	63	65
Circ	VII	24,291	21 603	17 458	14 317	2 638	2 928	4 195	4,358	72	66
Resp	VIII	7 807	6 644	2 767	2 499	3 067	2 661	1 973	1 484	35	38
Digest	IX	3 595	3 607	1 631	1 617	813	813	1 151	1 177	45	45
Gen urin	X	3 698	1 932	1,281	809	713	339	1 704	784	35	42
Other dis		4 441	4 363	1 616	1 351	257	276	2 568	2 736	36	31
Unknown	XVI	355	430	355	430	–	–	–	–	100	100
Accidents*	XVII	2 871	1 969	2 395	1 067	40	15	436	887	83	54
Suicide	XVIII	1 077	405	1 068	391	–	–	9	14	99	97
Total		66 927	60 360	42 031	36 163	8 360	8 130	16 536	16 067	63	60

p = primary (underlying) cause
c = complication
c = contributory cause
* including homicide (p 35 males 21 females)

Table 31B Mortality from DM by sex and age 1965

Attained age	Death shares for DM (per thousand) 1961–63				Number of deaths 1965				Expected number of deaths from DM 1965			
	Dp		Dt		All		Dp		Dp		Dt	
	M	F	M	F	M	F	M	F	M	F	M	F
0– 4	–	–	–	–	1 093	828	1	–	–	–	–	–
5– 9	–	7	1	10	145	81	1	–	–	0 6	0 1	0 8
10–14	9	4	9	9	107	66	–	1	1 0	0 3	1 0	0 6
15–19	5	11	7	15	299	134	3	1	1 5	1 5	2 1	2 0
20–24	13	28	15	31	330	126	–	3	4 3	3 5	5 0	3 9
25–29	47	72	49	79	274	117	4	11	12 9	8 4	13 4	9 2
30–34	43	59	50	65	311	168	12	14	13 4	9 9	15 6	10 9
35–39	29	24	38	32	473	272	21	10	13 7	6 5	17 1	8 7
40–44	24	19	31	23	681	491	15	9	16 3	9 3	21 1	11 3
45–49	10	9	21	18	984	699	14	9	9 8	6 3	20 7	12 6
50–54	14	13	28	27	1 784	1 095	21	16	25 0	14 2	50 0	29 6
55–59	13	15	31	39	2 581	1 606	26	28	33 6	24 1	80 0	62 6
60–64	12	23	35	63	3 820	2 342	45	64	45 8	53 9	133 7	147 5
65–69	12	27	41	80	4 970	3 503	73	114	59 6	94 6	203 8	280 2
70–74	12	26	45	88	6 166	5 002	106	136	74 0	130 1	277 5	440 2
75–79	13	22	47	78	6 744	6,549	92	176	87 7	144 1	317 0	510 8
80–84	10	13	41	59	5 985	6 408	77	114	59 8	83 3	245 4	378 1
85–89	8	10	25	40	3 722	4 602	42	46	29 8	46 0	93 0	184 1
90–	4	4	15	16	1 562	2 074	12	17	6 2	8 3	23 4	33 0
Total	12	18	37	60	42 031	36 163	565	769	494 4	644 9	1 519 9	2 126 1

Number of deaths 1965 Dt 1 810 males 2 490 females

Expected number of deaths from DM 1965 calculated with death shares for DM observed during the period 1961–63

In Table 31B a standard calculation has been performed in order to compare the registered mortality from DM in 1965 with the data from our study for the period 1961–63 The main results are shown in Table 31C

The statistics from 1965 are not sufficient for definite conclusions The registered increase in mortality from DM (by 15 to 20 per cent as compared with the data for the period 1961–63) may in part be ascribable to chance fluctuations

Table 31C Age standardized comparison of mortality from DM in 1961–63 and 1965

Attained age	Observed number of deaths from DM				Expected number (standard 1961–63)				Quotient O E per cent			
	Dp		Dt		Dp		Dt		Dp		Dt	
	M	F	M	F	M	F	M	F	M	F	M	F
0 24	5	5			7	6	8	7	74	83		
25–49	66	53			66	40	88	53	100	131		
50 74	271	358			238	317	745	960	114	113		
75–	223	353			183	282	679	1 106	122	125		
Total	565	769	1 810	2 490	494	645	1 520	2 126	114	119	119	117

but it seems likely that the main reason is a more complete registration of DM in the death certificates Unfortunately, the published data do not permit an analysis of the increase in respect of Dt with a division by age or with geographical divisions (different counties rural and urban areas) Nevertheless the statistics from 1965 clearly support the results obtained concerning the incidence and prevalence of DM in Sweden (which are based on statistics from 1961–63)

The completeness and representativity of the data on mortality from DM

From the preceding analysis it has appeared that the magnitude of the *domicile gaps* can be assessed at about 15 per cent for males and about 20 per cent for females It is extremely unlikely that the prevalence of DM in Stockholm City (in groups by sex and age) is mentionably below the corresponding figures for the central belt [1] As already stated it cannot be ruled out with certainty that the prevalence of DM is in fact lower in the south western part of Sweden than in the rest of the country but it seems very difficult to accept that the real differences are as large as the statistical data indicate The circumstances obtaining in Gothenburg and Malmo (with populations at the end of 1962 numbering 411,000 and 238 000 or with suburbs included 503 000 and 250 000 respectively) are in many respects similar to those of Stockholm (population 802 000 with suburbs in Stockholm County included 1 133 000) A scrutiny of Tables 25–27 and Figs 8–13 gives strong support for the view that the lower figures found for the south western counties are wholly ascribable to underregistration

The magnitude of the *cause gaps*, in addition to the effects of the domicile gaps has been assessed at about 10 per cent for males and about 8 per cent for females These cause gaps are mainly ascribable to an underregistration of DM among deaths from neoplasms and violence

In the comparisons made, the observed death shares and death rates for DM (primary and contributory cause) have been utilized Hence, no correction has been applied for the general underregistration of deaths from DM all over the country As will be seen from the next section the remaining *age gaps* can be assessed at about 10 per cent of the observed numbers for males and about 19 per cent for females

The mortality data from the period 1961–63 can be regarded as normal and there are no indications of a quantitatively significant time trend in respect of the incidence of DM

The filling up of the domicile gaps can be regarded as a mere correction of the observations for the whole country to the level observed for the central belt The filling up of the cause gaps can be regarded as a mere correction of the observations to the level observed for primary causes of death other than neoplasms and violence The filling up of the age gaps

[1] If environmental factors play a role in the development of DM it would rather be thought —in view of the clinical picture of the disease— that the morbidity risks and the death rates for DM would be higher in the large cities than in the less urbanized areas

can be regarded as a mere correction of the observations to the level observed for ages below 80 (taking into account the existing excess mortality for diabetics) With these three corrections, the total observed figures for the prevalence of DM among deaths should be increased by about one third (35 %) for males and by about half (52 %) for females It should be stressed that these figures are based on the analysis of *registered data on deaths from DM* (primary or contributory cause) and that it is quite out of the question that there has occurred any overregistration of such deaths

Whether the data on DM as a primary or contributory cause of death are sufficiently complete for the central belt in respect of deaths from other primary causes than neoplasms and violence at ages below 80 cannot be decided on the basis of a statistical analysis In other words the remainder of the possible general underregistration cannot be evaluated and the data obtained with the corrections mentioned above must be regarded as minimum figures for the prevalence of DM (among deaths) However, it seems likely that the remaining underestimation (the general underregistration) must be fairly moderate

Morbidity risks for DM and its prevalence in Sweden

The knowledge of *the prevalence of DM among deaths* (by sex and age) does not give definite information concerning *the prevalence of DM among living persons* (by sex and age) since the factor death implies—or may imply—a selection Mortality among diabetics may deviate considerably from mortality among non diabetics (or mortality in the general population) In fact, we do know that mortality among DM patients treated at hospitals as well as among diabetics taking out life insurance protection is higher than mortality in the general population and among 'normal insured persons, there exist many investigations in these fields some of them for fairly recent periods of time (cf, for instance, Marks 1963, Hayward & Lucena 1965) However knowing the excess mortality among diabetics (by sex and age) it is possible to calculate on the basis of the prevalence of DM among deaths at a certain point of time, the prevalence of DM among living persons at that time (by sex and age) It is then also possible to calculate, on the basis of these prevalence figures, *the morbidity risks for DM* (by sex and age)[1]—provided

(a) that the morbidity risks do not change with time (or, more precisely have not changed in the period during which the deceased persons have been living under the risk of developing DM)

(b) that the excess mortality (by sex and age) does not change with time

(c) that the registration of deaths from DM is equally complete in all groups (by sex and age)

However deviations from the pure model in the three respects mentioned

[1] From the medico-genetic viewpoint the aggregate morbidity risk (up to a given age) is the most suitable measure of morbidity (cf Larsson & Sjögren 1954 Larsson Sjogren & Jacobson 1963) It gives the probability that a person will develop the disease if he lives long enough (i e is not rescued by dying from another cause)

are capable of being evaluated with sufficient accuracy at any rate, for all practical purposes

Selective factors connected with excess mortality

The calculations below refer to the period 1961–63 corrections for gaps connected with domicile cause and old age will be applied at the final stages Before presenting the results of the calculations some special points on the representativity of the period 1961–63 and the effects of changes in excess mortality may be mentioned

(a) There is a heavy excess mortality among persons becoming afflicted with DM at younger ages (juvenile diabetics) With regard to the *number* of deaths among diabetics however, the effect of this excess mortality is very slight, since the proportion of juvenile diabetics is rather insignificant (As can be seen from Table 23 in the 3 year period 1961–63 the number of deaths from DM below age 40 was 145 males and 107 females or 3 3 and 1 7 per cent respectively, of the total registered number of deaths from DM)

(b) Among juvenile diabetics—and to a great extent among persons becoming afflicted with DM at adult ages too—the excess mortality was markedly higher in the pre insulin era than it has been since the introduction of insulin in general therapeutic practice Further, if we restrict the comparison to patients showing the same severity of symptoms the excess mortality must have been reduced after the introduction in general therapeutic practice of penicillin and antibiotics (which occurred in Sweden around 1942 and 1948 respectively) since in earlier periods infective diseases influenza and pneumonia were on average more dangerous to diabetics than to the population in general (of the same sex and age)

(c) The diagnostic techniques—not least in respect of laboratory facilities—have gradually become more accurate and more easy to handle Both absolutely and relatively the numbers of doctors nurses and beds in hospitals have increased communications in particular the availability of motor cars have improved largely These and other similar factors have contributed to the effect that in the population of diagnosed diabetics there has occurred a gradual admixture of comparatively mild cases which would not have been included under the conditions prevailing before and during the Second World War

(d) On the other hand it should be remembered that an excess mortality from a certain disease will often imply a positive selection of survivors in so far as, relatively speaking there will be more deaths among persons who are severely afflicted with the disease than among persons who have the disease in a milder form at least this must in general apply to chronic diseases causing (or connected with) excess mortality [1]

(e) Among persons who died during the period 1961–63 there are very few diabetics who became afflicted before insulin therapy was available however if the earlier survival rates had been augmented by the use of insulin, this would have meant only a small addition to the number of diabetics in the popula

[1] Cf Prawitz 1954

tion of 1961–63, even in the absence of excess mortality, the great majority of persons who became afflicted with DM before 1923 would not have survived as long as until 1961

(f) The antiselection against the aberrant gene (or genes) which conditions (or may be assumed to condition) the occurrence of DM may have been reduced —by means of insulin therapy or, more generally, through reduction of the excess mortality, by means of advances in obstetrics (preventive maternity welfare, etc), and as a result of general voluntary birth control However the role of these changes must be quite insignificant in respect of the prevalence of DM and the number of deaths among diabetics juvenile diabetics are few and forty years is too short a time for genetic factors of this nature to acquire any effect worth mentioning

(g) Both during the period 1961–63 and in certain preceding years there were undertaken screening procedures in respect of DM Further as already mentioned the introduction of obligatory health insurance in 1955 may have caused an acceleration in the diagnosing of new cases of DM However since we are dealing here with *the registered prevalence of DM among deaths* and not with *the registered incidence of new cases of DM* these factors will not have any disturbing effects on the analysis

Minimum estimate from total death share

During the period 1961–63 DM was recorded for 4 67 per cent of all deaths in Sweden (males 3 62 per cent females 5 87 per cent) related to deaths from known causes the share of deaths from DM is 4 73 per cent (males 3 66 per cent, females 5 96 per cent) Since there are no reasons whatsoever for assuming that the excess mortality for diabetics in general was more heavy during this period than in preceding years, it can immediately be concluded that the total morbidity risk for DM must exceed 3 6 per cent for males and 5 9 per cent for females If the assessment of the gaps (connected with domicile, cause, and old age) is applied the figures should be increased by 35 and 52 per cent, respectively, and hence the total morbidity risk for DM must exceed 4 9 per cent for males and 9 0 per cent for females

Minimum estimate from death share above a certain age

Unless mortality among persons afflicted with DM is lower than mortality in the general population the death shares for DM above a certain age will always (under stable conditions) give a minimum figure for the total morbidity risk, if onset does not occur after the age in question the share will give a minimum figure for the aggregate morbidity risk up to that age These shares are shown in Table 32 for deaths above age 50 the figures are 3 9 per cent for males and [illegible] 3 per cent for females and for deaths above age 65 they are 4 1 and 6 6 per cent respectively (as compared with 3 7 and 6 0 per cent for all deaths from known causes) Applying the same gap correction as above we arrive at the minimum figures 5 5 per cent for males and 10 1 per cent for females

Assessment based on assumptions concerning excess mortality

In order to evaluate the prevalence of DM among living persons, we must make reasonable assumptions concerning the excess mortality (by sex and age) for diabetics There will in fact be certain possibilities of checking that these assumptions are consistent with the results from the mortality statistics since the aggregate morbidity risk up to a certain age cannot decrease with increasing age

The assumptions concerning the excess mortality at ages before say 40 or 50 will have a comparatively slight effect for the assessment of the total prevalence of DM in the population At higher ages say above 60 or 65 the excess mortality must be fairly moderate and at advanced ages say above 80 or 85, the excess mortality if any can be neglected in view of the obvious age gaps in the registration of deaths from DM Guided by the results obtained in Chapter III with regard to the excess mortality among DM patients admitted to the investigation hospitals, and the trends by age in the death shares and death rates for DM registered in 1961-63 we have selected as a working hypothesis a smooth series of mortality ratios (the quotients by sex and age between the death rate for DM and the general death rate), for simplicity the mortality *ratios* are assumed to be equal for males and females but since the general death rates are higher for males than for females this implies that the *absolute* excess mortality is assumed to be higher for males Intentionally the series of mortality ratios has been chosen in such a way that there should not be any exaggeration of the total number of diabetics in the population (i e the level of the mortality ratios is rather too high than too low) Because this smooth series (denoted A) does not fulfil the criterion that the aggregate morbidity risk shall be a never-decreasing function of age the calculations are also performed with a modified series (denoted B)[1]

The series are as follows

Attained age	Assumed mortality ratio for persons with DM	
	A	B
-24	5	5
25-29	5	12
30-34	5	9
35-39	4	4
40-44	3	2.5
45-49	2	1.5
50-54	1 6	1 4
55-59	1 4	1 3
60-64	1 3	1.25
65-69	1 2	1.2
70-74	1 15	1 15
75-79	1 1	1 1
80-84	1 05	1 05
85-	1	1

The assessment of *the prevalence of DM in the population* (average during the period 1961-63 hence representing the middle of 1962) is then arrived at by simply dividing the death shares for DM by these mortality ratios

In the assessment of *the aggregate morbidity risks for DM* the excess mortality must be taken into account in another respect too The diabetics who in a certain age group are eliminated by the excess mortality would have been living in the next age group (as diabetics since DM can be regarded as a perma-

[1] The modifications are restricted to the age interval 25-64 under 25 the registered numbers of deaths from DM is low (in total 32 males and 65 females)

nent disease) if mortality had been the same among diabetics as in the general population [1] Therefore the extra deaths among diabetics should be carried forward to the next age group and be included there (as diabetics exposed to the excess mortality prevailing in this age group) [2]

The results of the calculations are shown in Table 32 In addition to the basic absolute numbers—population (three times the average population in 1961–63 in thousands) and registered deaths from DM (total during the 3 year period 1961–63 primary or contributory cause)—the table shows the death rates for DM per 100 000 population the death shares for DM per 1 000 deaths (related to deaths from known causes and adjusted to division by attained age) Further the death shares for DM are shown for all ages above the lower class limit (above 0 above 5 above 10 etc)

On the basis of the assumed mortality ratios (series A and B) stated in the table the prevalence of DM per 1 000 population and the absolute number of diabetics in the population (at the middle of 1962 in hundreds) have been calculated as shown in the table Two series of data refer to Assumption A viz. one without correction for registration gaps (other than those related to deaths from unknown cause XVII) and the other with correction for the domicile and cause gaps already discussed A third series refers to Assumption B with correction for the domicile and cause gaps and in addition a correction for age gaps The correction for age gaps is made on the simple assumption that at ages above 75 the prevalence of DM cannot be lower than the prevalence obtaining in the age groups 75–79 for males and 70–74 for females [3] Since the assumed mortality ratios A and B are the same for ages above 65, the correction for age gaps will also be valid for the A series Fi-

[1] Strictly speaking this applies to generations not to simultaneously living persons but if the population is stationary the results will be the same For the assessments made here the deviations from stationary conditions are without significance

[2] At ages where the excess mortality from DM is high the prevalence of DM is low and general mortality is low Therefore the figures for the prevalence of DM at a certain age will be only slightly below the aggregate morbidity risk for DM up to that age (the relative under estimation being less than 2 per cent)

With sufficient accuracy the aggregate morbidity risk m_x up to age x can be calculated with the formula (D = deaths from DM T = total deaths P = population r = mortality ratio for diabetics x = age)

$$m_x = \frac{D_x}{T_x r_x \left(1 - \sum_{y=0}^{x} \frac{r_y - 1}{r_y} \frac{D_y}{P_y}\right)}$$

or

$$m_x = \frac{D_x}{T_x \; r_x} \left(1 + \sum_{y=0}^{x} \frac{r_y - 1}{r_y} \frac{D_y}{P_y}\right)$$

Here D_x/T_x is the share of deaths from DM and $D_x/T_x r_x$ is the prevalence of DM in the population In the factor between brackets D P is below 0 01 per cent up to the age group 45–49 and $(r-1)$ r is assumed to be at the most 0 2 for ages above 65 For all ages under 60 the product (without correction for registration gaps) is below 0 01 per cent and the maximum is 0 047 per cent (for females in the age group 75–79)

[3] In respect of diseases which are genetically conditioned (or for other reasons affect only a certain part of the population) and cause (or are connected with) excess mortality there may occur a decrease in prevalence (especially if the morbidity risk at higher ages is zero or low) However in respect of DM the incidence of onset of the disease at higher ages is comparatively large and the excess mortality is comparatively low

Table 32 Assessment of morbidity risks for DM and its prevalence in Sweden 1961–63

	Excess mortality	Sex	All ages	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90
				0 003 × average population mortality data for DM and 0 01 × number of living diabetics by attained age																		
3 × average population thousands		M	11 318	800	819	894	963	754	668	694	769	[illegible]	794	794	696	591	471	351	241	129	[illegible]	12
		F	11 368	757	773	850	922	730	649	686	758	814	783	788	721	636	534	422	295	163	66	19
Deaths from DM primary or contributory cause		M	4 359	–	–	3	5	10	35	44	48	62	60	131	227	363	561	751	933	736	316	74
		F	6 238	–	3	2	5	8	[illegible]	34	25	31	35	84	169	408	795	1 295	1 515	1 147	547	105
Death rate for DM per 100 000 population		M	39	–	–	0	1	1	5	6	6	7	8	16	33	61	119	214	388	570	633	623
		F	56	–	0	0	1	1	5	5	3	4	5	11	23	[illegible]	149	309	513	701	833	559
Death share for DM per 1 000 deaths*		M	37	–	1	9	7	15	49	50	38	31	21	28	31	35	41	45	47	41	25	15
		F	60	–	10	9	15	31	79	65	32	23	18	27	39	63	80	[illegible]	78	59	40	16
Ditto *with all higher age groups included***		M		37	38	[illegible]	[illegible]	[illegible]	38	38	38	38	38	39	39	40	41	41	40	34	25	17
		F		60	61	61	61	62	62	62	62	62	62	63	64	66	66	65	58	48	35	17
Assumed mortality ratio *per cent*	A	M F			500	500	500	500	500	500	400	300	200	160	140	130	120	115	110	105	100	100
	B	M F			500	500	500	500	1 200	900	400	250	150	140	130	125	120	115	110	105	100	100
Assumed excess mortality per thousand	B	M			2	2	4	4	12	10	5	4	2	3	3	5	8	7	8	7	0	0
		F			1	1	2	2	7	6	3	2	1	2	2	3	4	5	7	6	0	0
DM per 1 000 population uncorrected	A	M	13	–	–	2	1	2	10	10	10	11	10	17	22	27	34	39	43	40	[illegible]	17
		F	20	–	2	2	3	5	16	14	8	8	9	16	26	47	61	77	72	58	43	17
Ditto corrected for domicile and cause gaps	A	M	17	–	–	2	2	3	12	13	12	14	13	21	28	33	43	49	54	50	36	21
		F	26	–	3	2	4	7	21	18	11	10	11	20	34	60	78	99	92	74	55	22
Ditto corrected also for age gaps	B	M	17	–	–	2	2	3	5	7	12	16	17	24	30	34	43	49	55	55	55	55
		F	27	–	3	2	4	7	9	10	11	12	15	23	36	63	78	99	99	[illegible]	99	99
Aggregate morbidity risk per thousand	B	M		–	–	2	2	3	5	7	12	16	17	24	30	35	43	49	55	55	55	55
		F		–	3	2	4	7	9	10	11	12	15	23	36	63	[illegible]	99	100	100	100	100
0 01 × number of diabetics uncorrected	A	M	446	–	–	5	4	6	22	24	24	30	27	45	51	52	53	45	35	17	5	1
		F	697	–	6	5	9	12	35	32	21	[illegible]	22	42	63	100	108	108	71	31	9	1
Ditto corrected for *domicile and cause gaps*	A	M	562	–	–	7	5	7	27	30	31	38	34	57	64	65	67	57	44	22	6	1
		F	[illegible]	–	7	6	11	16	44	40	27	28	[illegible]	54	[illegible]	128	138	139	91	40	12	1
Ditto corrected also for [illegible] gaps	B	M	573	–	–	7	5	7	11	17	31	45	46	65	69	68	67	57	44	23	9	2
		F	921	–	7	6	[illegible]	16	[illegible]	22	27	33	[illegible]	61	87	133	138	140	[illegible]	54	22	6
Approximate random error per cent		M				[illegible]	45	[illegible]	17	15	14	13	13	9	7	5	4	4	3	4	6	12
		F			58	71	45	35	18	17	20	[illegible]	17	11	8	5	4	3	3	3	4	10

* Deaths from unknown cause (XVI) have been proportionately distributed over known causes within their group by sex and age

** For instance the figures entered in column 40 44 refer to all deaths at ages 40 or over (40-)

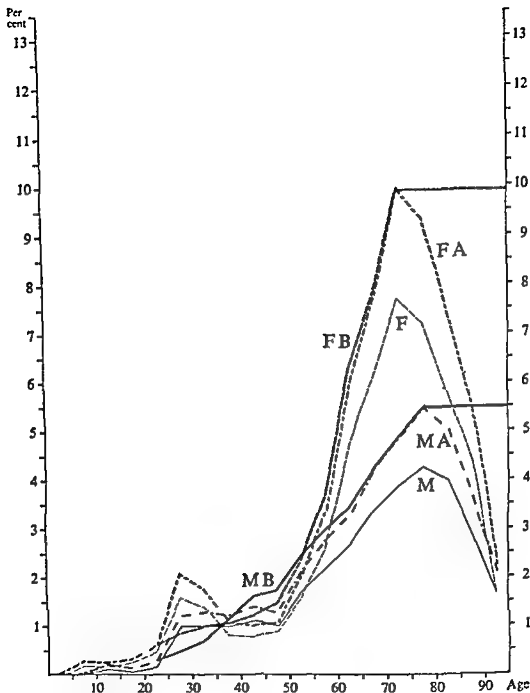


Fig 14 Assessment of the prevalence of DM in Sweden by sex and age 1961–63
See Table 32, p 111 and text pp 109–113

Table 33 Calculated number of diabetics in Sweden (average for years 1961–63)

Attained age	Population thousands		Calculated average number of diabetics, thousands							
			M				F			
			A			B	A			B
	M	F	u	d c	d c a	d c a	u	d c	d c a	d c a
0–49	2 661	2 574	14 2	17 9	17 9	16 9	16 4	20 8	20 8	18.2
50–64	694	715	14 8	18 6	18 6	20 2	20 5	26 3	26 3	28 1
65–79	354	417	13 3	16 8	16 8	16 8	28 7	36 8	37 6	37 6
80– •	64	83	2 3	2 9	3 4	3 4	4 1	5 3	8 2	8 2
Total	3 773	3 789	44 6	56 2	56 7	57 3	69 7	89 2	92 9	92 1

• Extrapolation of the morbidity risks indicates a further age gap (extrapolation gap) of about 0 3 for males and 3 2 for females

A B = assumptions concerning excess mortality (se Table 32)
u = uncorrected data
d c = corrected for domicile and cause gaps in registration
d c a = corrected for domicile cause and age gaps in registration

nally the table gives the calculated aggregate morbidity risks with Assumption B, as will be seen, the figures are practically identical with the prevalence series (corrected for domicile cause and age gaps) [1]

For the sake of simplification the corrections for the domicile and cause gaps have been made proportionately over all age groups As will have appeared already, the domicile gaps are positively and the cause gaps negatively correlated with age However, since the assumptions in respect of the excess mortality at younger ages are rather arbitrary, and the number of registered deaths is low, it has not been considered justifiable in the present connection to apply correction factors which vary with age

The relative *random* errors of the calculated prevalence figures are approximately proportional to the inverted square root of the registered number of deaths for convenience, these latter quantities are shown in the table Of course, the *systematic* errors (due to erroneous assumptions concerning the excess mortality) may be quite considerably larger

The results from the assessment of the prevalence of DM in the population are illustrated in Fig 14

Number of diabetics in Sweden

A summary of the calculations concerning the number of diabetics in the Swedish population (average 1961–63) is given in Table 33 As can be seen from the table, the difference between Assumptions A and B is of little importance for the assessment of the number of living diabetics

Fig 14 gives a strong impression that it must be unrealistic to accept that the aggregate morbidity risk for DM does not increase after the age of 80 Because of the unquestionable age gaps (already taken into account) it is not

[1] As already mentioned the relative difference between the aggregate morbidity risk and the prevalence is always less than 2 per cent

possible, on the basis of the mortality statistics alone, to arrive at definite conclusions concerning the rise of the aggregate morbidity risk for DM at advanced ages From our hospital series and from other data we know that *onset* of DM may sometimes occur at ages above 80, according to Table 5 this applies to 1 1 per cent of the patients in the hospital series but since the admission rate decreases markedly with age (at least in the higher age groups) the real share of very late onset must be considerably greater An extrapolation of the curves for the morbidity risks indicates that at age 90 the aggregate morbidity risks are at least 6 5 per cent for males and about 13 per cent for females This would mean that the correction for age gaps made in Table 33 should be increased by about 300 for males and 3 200 for females giving—with Assumption B—a total number of diabetics of 57 600 males and 95 300 females in the average population of 1961 63 or 1 53 and 2 52 per cent respectively of the population

It has sometimes been said that the prevalence of DM in the adult Swedish population is between 1 5 and 2 per cent such statements may be regarded as rough estimates (in part with the character of guesses) According to the analysis made here the figures for ages 15 and over are 1 90 per cent for males and 3 10 per cent for females and 2 50 per cent for both sexes taken together

It can be calculated that because of the changing age distribution (and the increase in population) the absolute numbers of diabetics will increase by 0 7 per cent annually for males and by 1 4 per cent annually for females At the end of 1965 there would thus have been about 159,000 diabetics in Sweden (59,000 males and 100 000 females), or 2 05 per cent of the total population (7 77 million)

For the sake of completeness reference may be made to some earlier investigations concerning the prevalence of DM in Sweden Most of the authors stress that their estimates should be regarded as minimum figures not infrequently, however the data are given in a form which may invite erroneous conclusions about the reliability of the results

On the basis of questionnaires answered by persons who received extra rationing cards during the Second World War Jorpes and Kallner (1942) recorded an overall prevalence of 0 163 per cent (rather plausible as a minimum figure)

A more extensive questionnaire was sent out in June 1943 On the basis of the answers and data concerning insulin consumption Dahlberg Jorpes Kallner and Lichtenstein (1947) calculated that in 1944 the number of diabetics in Sweden was at least 18 500 (or 0 28 per cent) this figure is regarded as a little too low For the City of Stockholm a special and more detailed inquiry was made the frequency of DM was found to be 0 506 per cent for males and 0 457 per cent for females By applying the Stockholm prevalence rates by sex and age to the whole country the total number of diabetics in Sweden was estimated to be 31 000 The authors conclude that in 1944 the number of diabetics in Sweden exceeds 22 500 but is less than 31 000 (or between 0 34 and 0 47 per cent) [1]

A Swedish governmental committee (1948) estimated the number of diabetics in Sweden to be between 35 000 and 40 000 (0 51–0 58 per cent of the total population) [2]

[1] Hanssen (1946) investigated the occurrence of DM in Bergen Norway during the period 1925–41 He estimated the prevalence of DM in 1941 at 0 38 per cent (0 32 per cent for males 0 42 per cent for females)

[2] 1943 års so-kersjukutrednings betänkande an gå nde so kersjukvården i riket *Statens Offentliga Utredningar 1948 33* Sto kholm 1948 pp 1–191

In Kristianstad County (L) in the south of Sweden Silver (1958) made a search for DM cases by means of hospital records and questionnaires sent to general practitioners (cf p 235), he registered an overall prevalence of 0 51 per cent

Using screening tests in connection with an extensive X ray examination of the population of Blekinge County (K) aged 10 years or over Schersten (1961) registered a DM frequency of 1 7 per cent applying corrections for certain gaps he arrived at the estimate 1 9 per cent (related to persons above 10 years of age) Related to the total population the corresponding figures would be 1 4 per cent and 1 6 per cent

Munke (1964) in a continuation of Schersten s study reports the prevalence of DM in the general population of Blekinge County to be 1 5 per cent

In his excellent popular book on DM and its treatment in adults Bo Andersson (1966) discusses Schersten s study and concludes that the data indicate an overall DM prevalence of 1 5 per cent In addition Andersson mentions preliminary results of a screening investigation in Norrbotten County (BD) which indicates a prevalence of 1 4 per cent However Andersson considers it probable that these figures are too low everyone working on DM is impressed by the fact that elderly persons may often be free from glycosuria in spite of a considerably increased blood sugar level A net to catch diabetics which is made of yarn from urinalysis alone will according to Andersson always prove to be too coarse meshed and therefore many diabetics will escape detection in this type of screening procedures

It is interesting to note that these Swedish figures are in good agreement with statistics and estimates for corresponding periods from other parts of the world (cf Schliack 1965 cf also Spiegelman & Marks 1946 Marks 1947 1961a Fox 1952, Newill 1963)

Fisher and Vavra (1964) report the prevalence of diagnosed (known) DM in the USA in 1959-61 to be 0 98 per cent (males 0 92 females 1 04) In addition according to their estimates the rate of undiagnosed (undetected) DM is 0 81 per cent These latter data are also given by age (but not by sex) a comparison with our results is shown in the following table

Attained age	Prevalence of DM per 1 000 population			
	Sweden		United States	
	Uncorr	Corr	Diagn	Total
0–24	1 7	2 1	1 0	1 7
25–44	10 7	10 6	5 0	10 2
45–54	12 9	19 9	15 2	33 1
55–64	30 2	40 5	31 4	55 6
65–74	53 0	67 8	42 4	68 6
75–	49 2	90 2	37 5	62.0
All	15 1	20 2	9 8	17 9

* Calculated from statistics on causes of death 1961-63 (Tables 32 and 33)

Uncorr Assumption A concerning excess mortality no correction for registration gaps

Corr Assumption B concerning excess mortality corrected for domicile cause and age gaps in registration

** Source Fisher and Vavra (1964) *Public Health Service Publication* No 1168

Diagn Diabetes reported in interviews July 1959-June 1961 Division of Health Statistics National Center for Health Statistics U S Public Health Service

Undiagn Estimates prepared on basis of studies and surveys by Diabetes and Arthritis Program Division of Chronic Disease U S Public Health Service

Total Diagn + Undiagn

As will be seen from the table the prevalence figures given by Fisher and Vavra for *diagnosed* DM are much lower than our *corrected* data and a good deal lower than our *uncorrected* data (both of which are based on death certificates and hence refer to diagnosed DM) Their total prevalence figures including undiagnosed DM agree very well with our results for the age group 65–74 for ages 45–64 their estimates are higher and for ages 75 and over they are appreciably lower than our figures

Excess mortality

With the age composition of the population in 1961–63 the assumptions would imply an overall excess mortality of 14 per cent (16 per cent for males 13 per cent for females) A comparison between the registered (observed) numbers of deaths from DM and the expected numbers is shown in the table below

Seen against the background of statistics from hospitals and insurance companies the assumption of an average excess mortality for diabetics (all ages taken together) of 14 per cent may appear too low Nevertheless irrespective of any theories concerning the magnitude of the excess mortality we know that the prevalence of DM at ages above 65—corrected for domicile and cause gaps, and corrected for unquestionable age gaps at ages above 75 (to the level found for males at ages 75–79 and for females at ages 70–74)—must be at least 5 5 per cent for males and 10 1 per cent for females An assumption that the average excess mortality at ages above 65 would exceed 14 per cent is not consistent with these data If the excess mortality at higher ages was greater than was assumed for the calculations in Table 32, this would imply that the age gaps must be larger, the estimated number of diabetics at these ages would remain approximately unchanged Modified assumptions concerning the excess mortality at ages below 65 will change not only the estimated numbers of diabetics but also the curves for the aggregate morbidity risks, and as can be seen from Fig 14 there is not much room for such modifications Whatever reasonable alterations are made, they will not greatly affect the results concerning the total number of diabetics

Taking into account the random errors, the curves for Assumption B in Fig 14 meet the criterion that the aggregate morbidity risk shall be a non-decreasing function of age It is possible to elevate the curves a little between ages 40 and 60, and it is possible to lower the curves a little for ages under 45 This means that the excess mortality in the four age groups 40–44 45–49, 50–54 and 55–59 may be *less than* the 150 50, 40 and 30 per cent respectively included in Assumption B, and that the excess

Attained age	Number of deaths from DM				Mortality ratio O E	
	O		E			
	M	F	M	F	M	F
0–49	267	173	92	51	2 92	3 43
50–64	721	661	578	537	1 25	1.23
65–79	2,245	3 605	1 993	3,204	1 13	1 13
80–	1 126	1 799	1 092	1 745	1 03	1 03
Total	4,359	6 238	3 755	5 537	1 16	1 13

O – Observed number of deaths from DM (primary or contributory cause) 1961–63

E – Expected number of deaths from DM calculated from the uncorrected numbers of living diabetics (assessment according to Assumption B not given in Table 31) and the death rates for the general population 1961–63 (Table 22)

mortality in the age groups 25–29, 30–34, 35–39 and 40–44 may be *more than* the 1 100, 800 300 and 150 per cent included in Assumption II For ages below 25 there are very few observations (36 registered deaths from DM, 18 males [illegible] females) the curves for the aggregate morbidity risks allow assumptions about the excess mortality in the interval 0–1 000 per cent

It has already been stressed that when general mortality is low, the relative excess mortality may appear to be great although the difference between the mortality of diabetics and that of the general population is fairly moderate In Table 32 are shown the *additions* to general death rates which are implied in Assumption II

The insurance problems will be discussed in Chapter VIII

Morbidity risks for DM

According to the foregoing analysis the aggregate morbidity risk for DM up to age 50 can be estimated at 2 per cent (possibly a little more for males and a little less for females) On the whole, the observations for ages below 50 do not indicate any irregularities with regard to the distribution by onset (cf Fig 14 and Table 32)[1] Hence for ages below 50 the average annual morbidity risk (the probability of becoming afflicted with DM) may be estimated at 0 4 per thousand After age 50 the morbidity risks are markedly higher in particular for females with increasing age the risk for females rises in the age interval 50–64, and then goes down again A—somewhat simplified—summary of the change of the morbidity risks with age is given in Table 34

Table 34 Morbidity risks for DM

Attained age	Annual morbidity risk for DM per thousand (graduated averages)		Age	Aggregate morbidity risk for DM per thousand (graduated figures)	
	M	F		M	F
–49	0 4	0 4	40	12	11
50–54	1 3	2 0	50	21	19
55–59	1 3	3 6	55	27	30
60–64	1 3	4 4	60	34	48
65–69	1 3	4 0	65	41	70
70–74	1 2	3 0	70	47	90
75–79	1 1	2 4	75	53	105
80–84	1 0	1 6	80	58	117
85–89	0 4	1 0	85	63	125
90–			90	65	130

In order to avoid misunderstanding, it should be emphasized again that the data presented here are wholly based on

(a) reports of deaths from DM (primary or contributory cause) in the death certificates

(b) analysis of probable gaps in these reports, connected with underregistration in certain geographical areas (mainly Stockholm City and the south western part of Sweden), with underregistration for certain primary causes of death (mainly neoplasms and violence) and with underregistration among deaths at advanced ages (mainly above 80) and

(c) reasonable assumptions concerning the excess mortality among diabetics

Hence the results refer to instances of *clinically diagnosed diabetes* and to instances of diabetes which—if not clinically *diagnosed*—are of a similar type

[1] There are in fact observations (from other fields) about such irregularities for instance connected with puberty but their quantitative effects can be neglected here

Quite naturally mild diabetic symptoms which do not in any noteworthy degree interfere with normal somatic functions will often escape registration What is discussed here is the degree of changes that is ordinarily found in clinically diagnosed DM

During the last decades medical progress has been great as regards most diseases of the younger ages but has been (though not inconsiderable) less marked in respect of diseases of old age There are reasons for assuming that prophylactic and therapeutic measures as well as medical research will in the future be directed to a greater extent than now to the needs of the older age groups and that this will affect both morbidity and mortality from disease The prospects of a continued reduction of morbidity and mortality at all ages—and consequently for males at advanced ages also—may be estimated as favourable There is nothing in the statistical results that argues against the hypothesis that at all ages there will be a decrease in morbidity and mortality from each of the major groups of causes—diseases of the circulatory system neoplasms and diseases of the nervous system and sense organs (cerebrovascular disease) For many reasons it seems warrantable to estimate that during the next few decades there will occur a decline in mortality in the older age groups that relatively speaking will be greater than the decline that took place in the first two decades after the end of the Second World War (cf Larsson 1965) However it does not seem likely that such changes *per se* will appreciably influence the prevalence of DM (in a certain group by sex and age)

It is not unreasonable to expect that the intensive research in different fields that is being carried on in respect of DM may result in a postponement of the onset of the disease (if not in a prevention) and then there will occur a reduction of the prevalence at least at younger ages and in the middle ages and a displacement "to the right" of the curves for the aggregate morbidity risk

Nevertheless the changing age structure of the population will inevitably be accompanied by an increase in the number of deaths from DM Whatever reasonable assumptions are made concerning a decline in mortality and a postponement of the onset of DM, this number will increase from about 5,100 per annum in 1961–63 (primary and contributory causes, corrected for registration gaps) to more than 6 000 per annum around 1980 unless the introduction of new prophylactic or therapeutic measures causes special delay effects the number of deaths from DM (primary and contributory causes) will exceed 7 per cent of all deaths

Life-table expectancy for DM

Often where estimates concerning the prevalence of DM are made they include not only persons afflicted with DM ("diagnosed" as well as "undiagnosed") but also persons thought likely to become afflicted with DM later on (unless "saved" by dying before the onset of DM)— *prediabetics* To avoid misunderstanding it should be emphasized that the statistics presented here do not include prediabetics

The calculation of the aggregate mor-

Table 35 Life table expectancy for DM

Age	Life table expectancy for DM up to age stated, per cent	
	M	F
25	1.1	1.0
50	2.0	1.9
55	2.5	2.9
60	3.1	4.5
65	3.6	6.5
70	4.1	8.1
75	4.4	9.2
80	4.7	9.9
85	4.8	10.1
90	4.8	10.2
Whole life	4.8	10.3

bidity risks presented above may be said to include prediabetics, hence, the total prevalence of diabetics and prediabetics in the Swedish population can be estimated at about 6.5 per cent for males and 13 per cent for females. However, a great many of these persons will never develop DM because of death before the onset of the disease. An expression of the probability that a newborn child will become afflicted with DM during his lifetime is arrived at by determining *the life table expectancy for DM*. The calculation shown in Table 35 is based on the life tables for the Swedish population for the 5-year period 1960–64.

Inferences from the study of death certificates

Mortality statistics which are based on data concerning both primary (underlying) causes of death and contributory causes can be utilized for estimating the prevalence and incidence of DM in the general population. At least in the case of Sweden, the analysis of such mortality statistics seems to be the most efficient method for determining the morbidity risks for DM and the prevalence (at adult ages) of clinically manifest DM by sex and age.

Mortality statistics which are based on data concerning primary causes of death alone, on the other hand, are not sufficiently informative (in respect of DM). The number of deaths for which DM is given as the primary cause does not cover more than 30–35 per cent of the total number of deaths for which the occurrence of DM is stated in the certificates (as the primary cause of death or as a contributory cause). The percentage varies markedly with age, it shows considerable geographical variations and, in addition, considerable variations with time.

A special processing of data from death certificates for the 3 year period 1961–63 was performed and the resulting statistics were analysed in detail with regard to sex, age, the primary cause of death, and domicile. The period 1961–63 presented normal mortality (viewed against the trend) and it cannot reasonably be suspected that the data are disturbed by any acceleration or delay effects.

There exist gaps in the recording of DM in the death certificates, connected with variations of the completeness of the registration by age, primary cause of death and domicile. In the main, however, these gaps are capable of being evaluated—apart from the possible occurrence of a general underregistration irrespective of age, cause, domicile and time.

The prevalence of DM recorded in the death certificates is lower in Stockholm City and in the south western part of Sweden (which includes the cities of Gothenburg and Malmo) than in other parts of the country Apart from the (rather unlikely) possibility of a lower prevalence of DM in south western Sweden, the statistics give clear evidence that the total morbidity risk for clinically manifest DM (the aggregate morbidity risk up to age 90) must exceed 5 5 per cent for males and 10 per cent for females With reasonable assumptions concerning the age gaps occurring at advanced ages and the excess mortality among diabetics, the best estimate seems to be that *the total morbidity risk for clinically manifest DM* (the risk up to age 90) is *at least 6.5 per cent for males and about 13 per cent for females* The aggregate risk up to age 50 is 2 1 per cent for males and 1 9 per cent for females, and up to age 70 it is 4 7 per cent for males and 9 0 per cent for females

The life table expectancy at birth for contracting clinically manifest DM is calculated at *4 8 per cent for males* and *10 3 per cent for females*

At the end of 1965 *the number of persons with clinically manifest DM* must have been *about 159 000* (59 000 males and 100 000 females) or 2 05 per cent of the total population (1 52 per cent for males, 2 57 per cent for females) For ages 15 and over the percentage is 2.60 (1 95 for males 3 24 for females)

It should be emphasized that all these data apply to *clinically manifest DM* and are wholly based on reports of deaths from DM (primary and contributory causes) in the death certificates, analysis of probable gaps in these reports (connected with underregistration in certain geographical areas, for certain primary causes of death and for deaths at advanced ages) and reasonable assumptions concerning the excess mortality among diabetics

The overall excess mortality among persons with clinically manifest DM cannot reasonably exceed 15 per cent (which, however, does not preclude that at ages below 50 the average excess mortality can be 200 per cent or more)

In our opinion the results reported in this chapter with regard to the morbidity risks for DM and its prevalence in Sweden at adult ages are to be considered as adequate and very reliable [1]

[1] Of course it cannot be ruled out on the basis of mortality statistics alone that in reality the prevalence of clinically manifest DM may be *still higher* Hence from a purely theoretical point of view our results should be designated as giving minimum figures. As will have appeared from the preceding discussion however, the general underregistration can be expected to have been comparatively moderate

CHAPTER V

Patients, symptoms and complications

As already stated the material for our clinical study covers 3 759 patients—in-patients as well as outpatients—registered at the four county hospitals of Vänersborg (P) Växjo (G) Falun (W) and Östersund (Z), in the main over the period 1931–56 All these patients presented both glycosuria and hyperglycaemia

Age at onset

Data on the age at onset of DM are included in Tables 5 and 7 The age distributions must of course be seen against the background of the size and age composition of the population in the admission areas further, it should be taken into account that our series comprises only patients admitted to departments of internal medicine As a concentrated description of the series certain data on age at onset are shown in Table 36 In addition to the absolute numbers of patients by sex hospital and types by age at onset (juvenile 0–14 early adult 15–39 late 40 and over) there are given by sex and hospital the percentage distributions by age at onset Further, the mean age at onset is shown by sex hospital and types by age at onset

The types with juvenile early adult and late onset cover 11, 27 and 62 per cent of the males and 8 15 and 77 per cent of

Table 36 Age at onset of DM a\erages and percentage distributions

Sex and age at onset	Number of patients					Percentage distribution by age at onset					Mean age at onset				
	All	Hospital				All	Hospital				All	Hosp .al			
		P	[illegible]	W	Z		P	G	W	Z		P	G	W	Z
Males															
0–14	183	50	46	51	36	10 9	12	15	11	7	8 8	8 5	8 3	9 0	9 6
15–39	4*0	117	77	153	103	26 8	29	25	33	21	27.2	27 7	26 4	27 0	27 5
40–	1 047	241	181	266	359	62 3	59	60	56	72	58 5	57 9	59.3	54 9	61.2
Total	1 680	408	304	470	498	*100 0*	*100*	*100*	*100*	*100*	44 7	43.2	43.2	40 8	50 5
Females															
0–14	169	42	40	55	32	8 1	9	9	10	5	8 5	8 1	8 8	8 4	8 9
15–39	319	79	66	111	[illegible]	15 3	17	[illegible]	20	10	26 7	26 5	26.5	27 0	26 9
40–	1 591	338	327	400	526	76 6	74	76	70	85	60 4	59 8	60 7	58 0	62.5
Total	2 079	459	433	566	621	*100 0*	*100*	*100*	*100*	*100*	51 1	49.3	50 7	47 1	56 2

the females The shares for late onset are considerably higher at Östersund (Z) viz 72 per cent for males and 85 per cent for females and they are somewhat lower at Falun (W), viz 56 and 70 per cent, than at Vänersborg (P) and Växjo (G)

The average age at onset is 44 7 for males and 51 1 for females It is higher at Östersund (50 5 and 56 2) and lower at Falun (40 8 and 47 1) while Vänersborg (43 2 and 49 3) and Växjo (43 2 and 50 7) occupy an intermediate position With regard to the three types by age at onset the figures are very similar in the juvenile onset and the early adult-onset groups, whilst certain differences similar to those found for the whole series are seen in the late onset group with higher average ages at Östersund and lower average ages at Falun Since the share of patients with late onset is also higher at Östersund and lower at Falun the differences become more accentuated for the whole series, irrespective of age at onset

Hyperglycaemia, glucose tolerance tests

In planning the study much time was devoted to discussing whether the blood sugar values registered should be included in the data processing and, if so how

The fasting blood sugar level is a valuable diagnostic criterion most textbooks agree that in healthy persons it should not exceed 0 11 per cent However when dealing with DM patients many of whom are treated with insulin or other antidiabetic remedies the blood sugar will often be influenced in a way that is very difficult to evaluate (at least quantitatively) It is a fairly frequent phenomenon that a well balanced DM patient (good control ') sometimes presents a high fasting blood sugar value although the treatment has been kept wholly unchanged, generally the explanation is that during the night the patient has had hypoglycaemia—of which he himself is unaware—and that this has started a strong reaction ('Gegenregulation) with secretion of glucagon resulting in considerable hyperglycaemia

Since for these reasons a statistical analysis of blood sugar values found in a series of the type studied here did not seem to be of any real significance, we decided to leave them out of the registration A classification of the patients on the basis of for instance, their average blood sugar value during a certain duration period could scarcely be expected to be efficient and so we limited the registration to cover *the occurrence of hyperglycaemia* (and hypoglycaemia)[1]

As a rule the blood sugar has been determined according to Hagedorn Jensen or, during the early thirties according to Folin-Wu The methods of Bang and Somogyi Nelson have been applied only exceptionally

According to Somogyi Nelson the known non glucose reducing substances are eliminated before the true blood sugar is determined However these non glucose reducing substances occur in the blood from diabetics and non diabetics in equal quantities (Haunz & Keranen 1950)

Hyperglycaemia has been considered to be present where the fasting blood sugar exceeds 0 11 per cent

[1] A study of blood glucose levels in adults in the U S A has recently been published by Garst (1966) Cf also Nilsson *et al* 1964 1967

Oral glucose tolerance tests (GTT) have been applied in doubtful cases

Several investigators have used glucose quantities of 1 to 1 75 grammes per kg body weight Others have recommended a dose of 50 to 200 grammes irrespective of the body weight In the present series a dosage of 1 gramme per kg body weight has as a rule been applied

Experience has shown that the results depend on the quantity of glucose to only a slight degree provided that it is not too small (below 25 grammes) Generally, a series of blood sugar tests are taken viz. after 30 45 60 75, 90 120 and 180 (sometimes also 240) minutes

On the evaluation of the results, special attention has been paid to the preceding fasting value and particular importance has been attached to the 2 hour value If the test is to be considered normal, this must not exceed the fasting value

Care has been taken to ensure that the patient shall have eaten a sufficient amount of carbohydrates during the days before the test

The oral GTT has been the topic of many discussions and varying technical procedures have been employed It has been argued that the test may be ameliorated by giving the glucose dose in two parts with half an hour s interval Fajans and Conn (1954 1961 1965) have administered cortisone before supplying the glucose in order to increase the reliability of the test

The intravenous GTT seems to have been introduced by Jorgensen and Plum in 1922 Apparently however it was difficult to arrive at a generally accepted interpretation of the results and so the method was abandoned It was reinvestigated by Hamilton and Stein in 1942 and during the last ten years there have been many studies of the techniques of the intravenous GTT and its diagnostic value for instance by Ikkos and Luft (1957) and Lunell (1966)

Hamilton and Stein (1942) showed that within 25–60 minutes after the injection of 20 grammes glucose intravenously the blood sugar values decreased exponentially with time (hence that in this interval the logarithms of the blood sugar values were a linear function of the time) this rate of removal of glucose k is lower among diabetics than among non-diabetics If the blood sugar value is denoted by C and the time (in minutes) after the injection by t the percentage rate k is determined from the formula

$$k = -100 \frac{d(\ln C)}{dt}$$

In healthy subjects k is below 1 0 (A k value of 1 0 corresponds to a reduction of the blood sugar value to one half in 69 3 minutes)

Quite apart from other aspects it is of course very convenient to be able to use a concentrated measure such as the quantity k in the analysis of blood sugar data

Samols (1965) has shown that after the injection of 25 grammes glucose there is found a marked increase of the insulin content in the blood plasma this indicates that the value of k is an expression of the quantity of insulin that can be mobilized

Unfortunately the intravenous GTT is not physiologically adequate as in the case with the oral test Injected directly into the blood, the glucose does not pass through the stomach and the intestinal mucous membrane as glucose and all its mother substances do under physiological conditions In a sense the ultimate purpose of studies of the carbohydrate metabolism should be to arrive at information concerning the ability of the body to utilize carbohydrates when making use of all available resources and in this respect the oral test seems to be more appropriate than the intravenous one

Dupre and Beck (1964 1966) have shown that an agent obtained from porcine duodenal mucosa biologically distinguishable from secretin and pancreozymin causes a significant reduction in the rate of glucose

disappearance in healthy men This material contained no immunologically active insulin The results support the theory that humoral secretion of the intestines is involved in the normal response to ingestion of glucose

It will be seen to be a clear disadvantage of the intravenous GTT that factors of this kind cannot be taken into account

Intravenous GTT has been used in our hospital series to only a comparatively small extent Therefore, a discussion of the value of this test in clinical practice would fall outside the scope of the present study

A review article covering the recent literature on the release of insulin as mediated by secretin glucagon and other gastrointestinal hormones has recently been published by Jorpes and Mutt (1967)

Glycosuria, "control"

In the literature especially in papers from the Joslin clinic, there are often detailed data on different degrees of con trol As a rule both the blood sugar level and the urinary excretion of glucose are taken into account Use is made of such expressions as excellent control good control fair control, poor control

The problems concerning the degree of control have in a sense become more complicated during recent years as already mentioned it has been shown that the fatty tissue plays an important role in respect of the DM metabolism During the period from which our material originates the treatment of DM was largely directed to the elimination of hyperglycaemia and glycosuria As a rule there are in the case records entries concerning the 24 hour urinary excretion of glucose, the average of these values for admissions (occasions) during each 5 year duration period has been taken as an expression of the degree of control obtained in the period In view of the character of the registrations a more exact parameter of a quantitative nature cannot be applied According to the averages mentioned the registrations (duration cards) have been classified into three types viz

Degree of control	Urinary excretion of glucose per 24 hours
Good	Not exceeding 25 grammes
Fair	26–50 grammes
Poor	Exceeding 50 grammes

Using this terminology, Tables 37 and 38 show the percentage distribution by control ' (glycosuria) within groups by sex, age at onset and duration and—for durations 0–4—within groups by sex age at onset and hospital

To avoid misunderstanding it should be stressed that the expression control is not applied here to give information concerning the time intervals between the patient s visits to the hospital but is solely intended to characterize the status of the patient in respect of the occurrence of glycosuria

As will be seen from Table 37 the control has been better among patients with late onset than among patients with juvenile or early adult onset In the last-mentioned groups the control seems to have been somewhat better in the higher durations than during the first 5 or 10 years after the onset of DM and somewhat better among males than among females In the late onset group on the

Table 37 Percentage distribution by "control" (glycosuria) within groups by sex age at onset and duration

Sex and duration	Juvenile onset (0 14) No	Control ( ) Good	Fair	Poor	Early adult onset (15 39) No	Control ( ) Good	Fair	Poor	Late onset (40) No	Control ( ) Good	Fair	Poor
Males												
0- 4	133	47	28	25	386	46	31	23	944	61	28	11
5- 9	126	46	25	29	252	51	29	20	469	66	21	13
10-14	121	54	31	15	173	60	23	17	212	64	22	14
15-19	87	60	22	18	124	64	23	13	101	66	27	7
20-24	48	56	21	23	71	70	17	13	37	57	26	16
25-	33	76	18	6	69	66	25	9	12	92	–	8
Total	548	53	26	21	1 075	54	27	19	1 775	63	25	12
Females												
0- 4	125	47	35	18	251	40	36	24	1 423	72	21	7
5- 9	115	44	35	21	186	46	31	23	782	67	24	9
10-14	114	51	27	22	131	46	37	17	340	69	24	7
15-19	68	47	40	13	88	53	34	13	142	72	22	6
20-24	37	54	38	8	58	60	30	10	33	73	21	6
25-	26	73	12	15	58	74	19	7	10	80	20	–
Total	485	49	33	18	772	48	33	19	2 730	71	22	7

Urinary excretion of glucose per 24 hours — Good control: not exceeding 25 g; Fair control: 26–50 g; Poor control: exceeding 50 g

Table 38 Percentage distribution by "control" (glycosuria) within groups by sex, age at onset and hospital duration 0–4

Sex and hospital	Juvenile onset (0 14) No	Control ( ) Good	Fair	Poor	Early adult onset (15 39) No	Control ( ) Good	Fair	Poor	Late onset (40-) No	Control ( ) Good	Fair	Poor
Males												
P	47	58	19	23	115	55	27	18	234	67	23	10
G	33	36	18	46	66	47	12	41	165	71	15	14
W	23	35	30	35	115	24	41	35	214	35	41	24
Z	30	50	50	–	90	60	39	1	331	67	31	2
Total	133	47	28	25	386	46	31	23	944	61	28	11
Females												
P	39	43	44	13	73	45	26	27	336	73	22	5
G	28	43	21	36	42	52	19	24	286	82	10	8
W	31	23	55	22	82	27	47	26	312	52	10	8
Z	27	[illegible]	15	–	54	45	44	11	489	79	21	–
Total	125	47	35	18	251	40	36	24	1 423	72	21	7

other hand the control has been better among females than among males

From Table 38 it is evident that there exist marked differences between the four hospitals The registered frequency of poor control at duration 0–4 years is

higher at Falun (W) and Vãxjo (G) than at Vanersborg (P) It ıs very low at Öster sund (Z) or 2 per cent agaınst an average of 17 per cent for the other three hospitals, ın part this difference is due to the composition of the series, with comparatively many cases of late onset at Östersund The registered frequency of "good con trol is fairly similar at the hospitals of Vanersborg (P) Vaxjo (G) and Östersund (Z) but considerably lower at Falun (W) where on the other hand the figures for fair control' are comparatively high

Heredıty

It has long been assumed that there are important hereditary factors in DM, and therefore it has been a routine procedure to interrogate the patients and if possible their family members about the occurrence of DM among relatives

However the completeness of the en tries in the case records varies considerably At Vãxjo (G) both positive and negative answers have regularly been registered At Falun (W) "interesting information is carefully recorded, in particular where several family members and relatives have been reported as afflicted with DM, whereas in general less interesting information is omitted At Östersund (Z) the registration seems to have been very incomplete, and for this reason the material was supplemented by a special questioning of patients visiting the hospital in 1958 At Vanersborg (P), finally, interviews have been performed over a long period

The prevailing opinion among clinicians has been that hereditary DM is con ditioned by an autosomal recessive gene Hence it is natural that interest has been concentrated on the occurrence of DM among the sibs of the patients It is also understandable that the registration often does not give full information concerning the sex of the affected relatives and their exact blood relationship to the patients

Table 39 Diabetic relatives of DM patients at Vanersborg

	Male patients				Female patients			
	All	Age at onset			All	Age at onset		
		[illegible]	15 39	40		0 14	15 39	40
Number of patients	408	50	117	241	459	42	79	338
Ditto information available	388	48	115	225	421	36	71	314
Number of patients with DM among relatives								
Paternal	63	12	24	27	60	7	19	34
Maternal	70	12	23	35	70	6	10	54
Both sides	13	5	6	2	14	1	2	11
Total	120	[illegible]	41	60	116	12	27	77
Percentage of patients with DM among relatives								
Paternal	16	25	[illegible]	12	14	19	27	11
Maternal	[illegible]	25	20	16	17	17	14	17
Both sides	3	10	5	1	3	3	3	4
Total	31	40	36	27	[illegible]	33	38	24

for instance there are many data of the type child, 'two sibs, cousin, maternal cousin, ' brother s children etc

A summary of the data from Väners borg in respect of the information concerning DM among relatives other than sibs and children is given in Table 39 Answers were obtained for 388 of the 408 male patients and 421 of the 459 female patients, the percentages are calculated on the basis of these answers

DM among relatives other than sibs and children was registered for 31 per cent of the male patients and 28 per cent of the female patients The information is less complete for patients with late onset (27 and 24 per cent) than for patients with juvenile or early adult onset (37 and 36 per cent) On the paternal side DM was registered for 16 per cent of the male patients (for 13 per cent on the paternal side alone) and for 14 per cent of the female patients (for 11 per cent on the paternal side alone) On the maternal side (the maternal side alone) DM was registered for 18 (15) per cent of the male patients and for 17 (14) per cent of the female patients

For patients with DM relatives on the paternal side (63 males and 60 females) the mean number of paternal DM relatives is 1 3 For patients with DM relatives on the maternal side (70 males and 70 females) the mean number of maternal DM relatives is 1 2

These data seem to argue in favour of a theory of autosomal inheritance However it should be kept in mind that in several instances it is a question of comparatively distant relatives (for instance a paternal first cousin) The problem of the hereditary nature of DM will be subjected to closer scrutiny in Chapter IX, in this connection data from two of the investigation hospitals will also be presented

Overweight

The problem of overweight among DM patients has interested diabetologists for many years Both in his textbook *Treatment of Diabetes* and elsewhere, Joslin has emphasized that overweight plays an important role for the occurrence of clinical DM He points out that overweight precedes the onset of DM, after which there is often a loss of weight this may be either a result of a diet poor in calories or a consequence of the reduced ability of the body to metabolize the administered carbohydrates

The classification by overweight is performed by comparison with the normal weight according to Broca s formula W= H-100, where H is height (in cm) and W is weight (in kg) Overweight is considered to exist where the registered weight exceeds the normal value by 10 kg or more [1]

Data on the height of the patients are not always registered in particular there are gaps concerning the children Among patients aged 21-60 the mean values of the registered height are 173 8±0 34 cm for males and 161 4±0 25 cm for females These values are significantly below the figures found for the general Swedish population However, it seems likely that the data on height have been more completely registered in the case of low stature than in the case of average

[1] For patients under 20 years of age modified criteria were applied

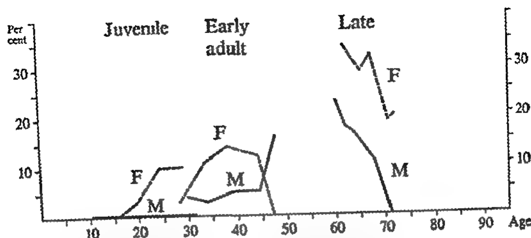


Fig 16 Frequency of obesity by sex, age at onset and duration
The plotting is made against the mean ages given in Table 41

skoga (in Örebro County T) With Broca s formula, overweight is considered to exist where the registered weight exceeds the normal value by 11 kg or more

Boe Humerfelt and Wedervang in their extensive study of the general population of the city of Bergen, Norway, registered the height weight and blood pressure of 9 783 men and 13 845 women this part of their series relates to 1951 The authors determined the 'normal weight with the formula W=0 00228 H² but since the presentation of the data is excellent it is easy to calculate the frequencies of overweight 10 kg or more according to Broca s rule

The data are illustrated in Figs 15 and 16

As can be seen from Table 40 and Fig 15 the three series show a rather similar picture in respect of males whereas in respect of females the frequency of obesity is lower among the DM patients than in the general population of Bergen and among the Karlskoga patients Evidently the DM patients do not present overweight to an extent that exceeds what is to be found in the general population or among 'ordinary' patients in a department of internal medicine Whether or not there has been a higher frequency of overweight among the DM patients *before* the onset of the disease is a question that cannot be determined on the basis of the registered data

Jorde (1961) has performed a comprehensive survey of DM patients in Bergen his results show overweight frequencies by sex and age which on the whole agree well with the figures found by Boe, Humerfelt and Wedervang for the general population

Constitutional type

As was to be expected the case records in respect of constitutional type proved to be very incomplete It is only natural that in a registration which is intended to show anamnestic data of relevance for the onset and development of a disease and clinical

data of importance for the treatment to be applied, "normal" findings should be omitted if they are not considered to be particularly interesting (or, for one reason or another, the registration is intentionally designed to include the data in question)

On the basis of the entries in the case records we have tried to differentiate between four body types viz (a) asthenic, (b) pyknic, (c) athletic, and (d) hormonal

The expression hormonal type here to denote patients who are m stature, often with a certain over and an infantile mentality—a which is not seldom seen in diabet dren to a certain extent these fe recall Cushing s disease[1]

The results of this classificatio patients with duration 0–4 yea

[1] In addition there are included in th type some patients with Turner s syndro

Table 42 Distribution by registered constitutional type

Sex and age at onset	Hospital	Number of patients (duration 0–4)						Percentage distributio (cut off)			
		Total	Constitutional type								
			Non specified	Asth.	Pykn.	Athl.	Horm	Non specified	Asth	Pykn.	Athl (cut off)
Males											
0–14	P	47	46	–	–	1	–	98	–	–	2
	G	33	32	1	–	–	–	97	3	–	–
	W	23	21	2	–	–	–	91	9	–	–
	Z	30	25	5	–	–	–	83	17	–	–
15–39	P	115	95	15	2	2	1	83	13	2	2
	G	66	47	15	2	1	1	71	23	3	3
	W	115	109	5	–	1	–	95	4	–	1
	Z	90	53	29	7	–	1	59	32	8	–
40–	P	234	190	[illegible]	22	12	–	81	4	9	5
	G	165	127	14	12	11	1	77	8	7	7
	W	214	202	8	3	–	1	94	4	1	–
	Z	331	232	33	60	6	–	70	10	18	2
	Total	1 463	1 179	137	108	34	5	81	9	7	2
	P G Z	1 001	744	116	105	32	4	74	12	10	3
Females											
0–14	P	39	37	–	–	–	2	95	–	–	–
	G	28	28	–	–	–	–	100	–	–	–
	W	31	25	2	1	–	3	81	6	3	–
	Z	27	24	1	1	–	1	89	4	4	–
15–39	P	73	64	4	3	1	1	88	5	4	1
	G	42	34	7	1	–	–	81	17	2	–
	W	82	79	3	–	–	–	96	4	–	–
	Z	54	30	16	7	–	1	56	30	13	–
40–	P	336	275	6	47	3	5	82	2	14	1
	G	286	231	13	41	–	1	81	5	14	–
	W	312	299	4	7	1	1	96	1	2	0
	Z	489	284	21	180	–	4	58	4	37	–

shown in Table 42 The data are given by sex, hospital and age at onset (juvenile, early adult, late) As will be seen from the table the information in the case records is not sufficient to allow a classification into one of the four body types mentioned for more than 19 per cent of the males and 22 per cent of the females It can further be seen that the data from the four hospitals differ markedly in several respects (which may serve as a warning against the use of case record data without a close scrutiny of the material) Falun (W) shows a considerably lower share of specified types than do the other three hospitals Östersund (Z) presents comparatively high percentages for the pyknic type (or, rather, a more complete specification)

Obviously it is difficult to draw definite conclusions from the data adduced in Table 42, but it might be said that they are consistent with current views that among diabetics the asthenic type preponderates at younger ages, whilst the pyknic type is frequently found in late onset diabetes

Blood pressure

In a way it is somewhat surprising that in general textbooks on DM little attention is paid to the questions of blood pressure and hypertension As a rule, statistical data on the distribution by blood pressure for different categories of diabetics are lacking

In *The treatment of diabetes mellitus* (Joslin Root White & Marble 1959) Root and Bradley write as follows (p 427)

The harmful effects of persistent arterial hypertension in the diabetic have long been known As is generally true systolic hypertension especially in females has proven less serious than elevation of diastolic pressure

The sequence of events is important (1) *Diabetes itself* produces hypertension of the *renal* type because of its influence on the premature development of arteriolar disease in the kidney (2) Diastolic hypertension whether (*a*) of the essential or 'primary type, (not caused by diabetes) or (*b*) produced by renal arteriolar disease (caused in part by diabetes) accelerates atherosclerosis

Mårtensson (1950) in a study of cardiovascular and renal findings in long standing DM, considers hypertension to be present when the systolic blood pressure exceeds 150 mm Hg or the diastolic pressure exceeds 90 mm Hg However, in the processing of the data he uses the systolic pressure alone The series covers 110 males and 109 females aged 16–84 with a duration of DM 15–34 years Martensson found hypertension to be present for 48 per cent of the males and 65 per cent of the females

Lundbæk (1953), investigating long-term diabetes in a series of 164 patients, found hypertension in 48 per cent of the patients Hypertension was considered to exist where the systolic pressure was more than 150 mm Hg or the diastolic pressure was more than 100 mm Hg Lundbæk found significant differences between males and females with a higher prevalence of hypertension among females, especially those above 50 years of age, no correlation between the duration of DM (in the interval 15–25 years) and hypertension was found

In a study dealing with atrophic skin lesions in the lower extremities of diabetics Melin (1964) gives data on hyper

tension His series covers 293 patients (130 males and 163 females) aged 10–90, hypertension is defined as a diastolic pressure of 100 mm Hg or over The prevalence of hypertension increased with age but was not found to be connected with the duration of DM The prevalence was higher among females than among males at ages over 60 it was 57 per cent (47 out of 82) and 34 per cent (14 out of 41) respectively

At the departments of internal medicine in the Swedish county hospitals it is a routine procedure at least for in-patients for the blood pressure to be measured and registered With regard to diabetics who are examined or treated as outpatients it is a rule that as far as possible measurements and registrations of blood pressure should be performed There are certain gaps in the case records but a scrutiny has revealed that such gaps are generally found only for patients who at a later examination (or at an examination some weeks or months before) have had "normal blood pressure There are very few patients for whom no entry at all is found in a 5 year registration period (duration 0–4 5–9 etc) For these reasons absence of information during a registration period has been interpreted as representing "normal blood pressure

It is nowadays generally agreed that an evaluation of the occurrence of hypertension in DM patients should be based on the diastolic pressure

In transferring the case record data on diastolic pressure to the duration cards we used the arithmetic mean of the registrations during each admission and then again the arithmetic mean of these during each registration period At both these procedures the values were grouped into classes with the range 10 mm Hg This implies that on average the class limits are 75, 85 95, 105 etc which was considered advantageous because there was an obvious tendency to state the values in "whole tens" (70 80, 90, 100, etc)[1] Hence with the direct use of the single measurements there would have arisen a large difference between for instance the two frequency series 100 and over and over 100 The method adopted can be said to constitute a graduation which at least approximately eliminates the effects of the "rounding-off" in the primary data

In Table 43 data on diastolic blood pressure are shown by sex and age at durations 0–4 and 10–14 years The table shows the prevalence of diastolic pressures of at least 95 and at least 105 mm Hg The frequency of hypertension increases with age, at ages above 40 the frequency is higher among females than among males An interesting feature is that at ages above 40 the frequency of hypertension is significantly higher among patients with DM duration 0–4 years than among patients of the same age with DM duration 10–14 years It should be noticed here that in each of the two duration groups a patient is counted only once but that the greater part of the

[1] It may be noted that the same phenomenon is to be seen in the expert study on blood pressure in men aged 50 recently published by Tibblin (1967) Tibblin applied very strict rules for the measurements The blood pressure was read to the nearest 5 mm Hg more precise readings were considered to be of little value Nevertheless for 841 men the diastolic and systolic pressures were even multiples of 5 in no fewer than 467 cases (55 5 per cent) and 449 cases (53 4 per cent) respectively

patients with duration 10–14 are also included with duration 0–4

The frequency figures in Table 43 are illustrated in Fig 17

For the three types by age at onset (juvenile, early adult, late) data on the frequency of hypertension are given in Table 44 by sex and the duration of DM For juvenile onset there is seen an obvious rise in the frequency with duration, in particular from duration 5–9 to duration 15–19, here the age effect found in Table 43 must be insignificant For early adult onset the rise in frequency, which can be seen from the table, is fairly similar, but from Fig 17 it is apparent that the greater part of it is due to the age effect For late onset finally, the frequency is markedly higher at duration 0–4 years than at duration 5 years or over It might be suspected that this was due to peculiarities in respect of the age distribution, viz. that the patients with duration 0–4 are *older* than are the patients with duration 5 and over However, this is not the case, in Table 44 there is stated the mean age of the patients in each group by sex, age at onset and duration (below 25 years), as can be seen, the 'selection' in respect of age composition, arising from deaths and the gradually increasing admission of elderly patients, gives the result that from duration 0–4 to duration 5–9 the mean age goes up for males by 2 3 years (from 61 1 to 63 4) and for females by 1 8 years (from 63 1 to 64 9) Thus, there [illegible] a displacement in the age composition, since the mean age does not increase by 5 years, but there must be another type of selection too The data do not admit of a judgment being made between the three possible explanations, viz (1) that

Table 43 Frequency of hypertension in duration groups 0–4 and 10–14 by sex and age

Average age	Males								Females							
	Number with duration		Diastolic blood pressure per cent						Number with duration		Diastolic blood pressure per cent					
			Dur 0–4		Dur 10–14						Dur 0–4		Dur 10 14			
	0–4	10 14	95–	105–	95	105			0–4	10 14	95–	105–	95–	105–		
4	16		–	–					15		–	–				
9	44		–	–					56		4	4				
14	73	19	3	–	11	–			54	16	4	–	25	6		
19	78	48	3	–	17	13			53	56	9	2	21	7		
24	[illegible]	54	3	–	20	4			50	42	8	2	24	10		
29	79	36	8	–	19	8			43	24	15	3	13	13		
34	80	34	15	1	6	–			55	30	7	2	17	7		
39	81	35	15	5	23	3			50	21	18	4	10	–		
44	99	37	30	11	24	11			71	26	41	14	27	8		
49	111	31	36	11	19	13			116	30	57	28	43	13		
54	131	49	46	21	24	8			183	36	68	36	36	17		
59	154	33	53	27	42	18			249	51	63	38	55	35		
64	142	47	44	15	40	26			246	[illegible]	66	43	56	40		
69	125	43	57	28	42	28			261	86	72	42	55	29		
74	98	20	63	32	35	30			183	46	69	47	63	35		
79	65	16	55	29	25	13			94	25	74	41	48	28		
84–	19	4	21	11	–	–			20	11	75	30	36	27		
Total	1 463	506	33	14	25	12			1 799	585	54	31	41	22		

Per cent

(a) 95—

F 0—4

F 10—14

MB
FB2
FB1
MB1

M 10—14

M 0—4

70 60 50 40 30 20 10

10 20 30 40 50 60 70 80 90 Age

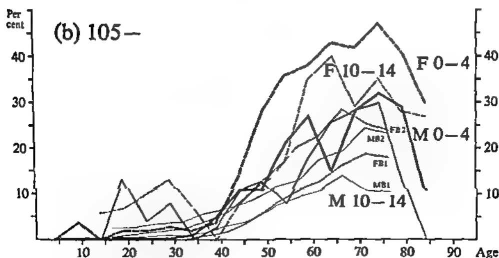


Fig 17 Frequency of hypertension by sex and age

(a) Diastolic blood pressure 95 or over
(b) Diastolic blood pressure 105 or over

B1 = Bergen Group 1 (1950) B2 = Bergen Group 2 (1951)

Table 44 Frequency of hypertension by sex, age at onset and duration

Age at onset	Duration	Males						Females					
		All	Mean age	Diastolic blood pressure				All	Mean age	Diastolic blood pressure			
				Number		Per cent				Number		Per cent	
				95–	105–	95–	105–			95–	105–	95–	105–
0–14	0– 4	133	11 1	2	–	2	–	125	10 6	4	2	3	2
	5– 9	126	16 0	7	–	6	–	115	15 7	12	2	10	[illegible]
	10–14	121	20 4	21	8	17	7	114	20 1	26	9	23	8
	15–19	87	25 0	20	11	23	13	68	23 0	23	9	34	13
	20–24	48	30 4	13	7	27	15	37	29 7	16	6	43	16
	25–	33		7	3	20	9	26		6	2	23	8
	Total	548		70	29	13	5	485		87	30	18	6
15–39	0– 4	386	29 2	33	4	9	1	251	29 0	28	6	11	2
	5– 9	252	34 8	24	7	10	3	186	33 5	[illegible]	5	16	3
	10–14	173	38 8	32	12	18	7	131	39 3	30	11	23	8
	15–19	124	42 8	33	15	27	12	[illegible]	46 2	35	15	40	17
	20–24	71	47 0	20	8	28	11	58	48 0	27	11	47	19
	25–	69		28	11	41	16	58		28	12	48	21
	Total	1 075		170	57	16	5	772		177	60	23	8
40–	0– 4	944	61 1	446	199	47	21	1 423	63 1	940	546	66	38
	5– 9	469	63 4	173	81	37	17	782	64 9	409	217	52	28
	10–14	212	64 4	74	42	35	20	340	66 6	181	109	53	32
	15–19	101	67 4	38	21	38	21	142	69 5	80	46	56	32
	20–24	37	70 9	11	7	30	19	33	70 8	16	9	48	27
	25–	12		4	1	33	8	10		4	3	40	30
	Total	1 775		746	351	42	20	2 730		1 630	930	60	34
All	Total	3 398		986	437	29	13	3 987		1,894	1 020	48	26

the treatment brings about a reduction of hypertension, at least for a certain share of the patients, (2) that there is an excess mortality among DM patients with hypertension and (3) that there is a selective admission in so far as among new cases of DM there is a greater probability of seeking aid at a county hospital if the patient is hypertensive than if he is not Very likely the frequency data are influenced by all these circumstances—and possibly their relative importance may be different for different parts of the series (the four investigation hospitals the distance from the patient s home to the hospital, and so on)

The data in Table 44 are illustrated in Fig 18 (for durations below 25 years), in order to take into account the age effect, the plotting is made against the mean ages given in the table

As stated already in connection with the discussion of obesity, the Bergen study by Boe, Humerfelt and Wedervang is not only thorough (as is often the case with epidemiological investigations) but also is published in a way that makes it possible for others to utilize the data after rearrangement (which is not the case with a great many other such investigations) From their data we have calculated the frequencies of diastolic blood pressure 95 and over, and 105 and over, in Fig 17 these data are compared with our

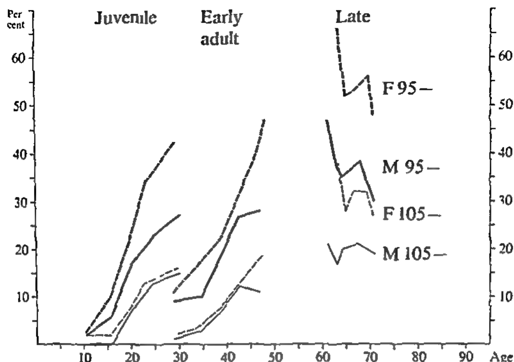


Fig 18 Frequency of hypertension by sex age at onset and duration

figures for DM patients as shown in Table 43

Tibblin s study of a representative sample of 50-year-old men in Gothenburg (1967) may be used for comparison As already mentioned (p 133) the blood pressure was read to the nearest 5 mm Hg The distribution according to diastolic pressure was as follows

Diastolic pressure	–80	85	90	95	100	105	110–	Total
Number	205	117	178	129	70	44	98	841

The frequencies of diastolic pressure 105 0 or over and 95 0 or over would thus be 14 per cent and 33 per cent respectively

Freedman Moulton and Spencer (1958) investigated the occurrence of hypertension (diastolic blood pressure of 100 mm Hg or more) in diabetic patients attending the University College Hospital Clinic in London and a control general hospital population According to the authors a strong impression was gained that hypertension was more common in diabetics than in the general hospital population Their statistical analysis revealed that this was largely a false impression up to 69 years of age in both sexes there was no significant difference in the hypertension frequencies By sex and age their data are summarized in the following table (see next page)

Although the frequency figures for hypertension (diastolic blood pressure 95 and over or 105 and over) may appear to be high it can be seen from Fig 17 that they agree fairly well with the frequencies existing in the general population The differences may in part be caused by the selective factors already mentioned and it does not seem justifiable to conclude from the data adduced

Attained age	Number of patients				Hypertensive per cent			
	M		F		M		F	
	G	D	G	D	G	D	G	D
0–29	188	98	244	98	1	–	–	–
30–39	180	70	227	68	1	1	4	1
40–49	178	74	236	111	5	7	10	6
50–59	147	105	239	211	14	16	23	17
60–69	98	65	161	133	23	31	36	37
70–	36	16	97	51	31	81	38	92
Total	827	428	1,204	672	8	13	15	21

G = General hospital population (controls)
D = Diabetic patients
Hypertensive = Diastolic blood pressure 100 mm Hg or more

After Freedman Moulton and Spencer (1958)

here that DM *per se* causes hypertension From a series of clinical studies it will appear that the specific renal complications of DM (diabetic nephropathy) do not give rise to hypertension until the pathological changes have progressed so far that renal insufficiency occurs

Menarche, childbirth, and menopause

Data on the age at menarche and menopause are not systematically included in the case records On the whole, the registration seems to have been restricted to cover the occurrence of late menarche and early menopause At Vänersborg (P) special interviews were made in 1956–60 for a series of unselected patients who visited the hospital for control during that period

Data on pregnancies before and after the onset of DM (or before and after the first admission to the hospital) and on the weights of the children are registered to a great extent but not in a form suited to statistical processing There are obviously gaps in respect of the registration of abortions, and often the mother's age at childbirth (or the age of the child) is not given for children born before the first admission to the hospital Therefore, the analysis will be restricted to the Vänersborg material, where so far as possible information has been assembled from earlier visits to other hospitals and supplemented by means of "retrospective" interviews

Menarche

At Vänersborg, interview answers were obtained from 147 patients, viz 27 with juvenile onset, 45 with early adult onset, and 75 with late onset In these three groups menarche at age 16 or over was registered for 56 per cent, 16 per cent and 32 per cent, the mean age at *menarche* was 15 9, 14 0 and 15 0, respectively For the 17 patients with age at onset below 10 the mean age at menarche was 16 3

In Sweden as in many other countries, a gradual lowering of the menarche age has occurred during the last few decades In evaluating the figures for early adult

and late onset DM this circumstance must be taken into account In respect of juvenile onset DM it is apparent that the menarche often occurs later than is the case in the general population

Menopause

In the Vänersborg series there are in all 338 female patients with late onset DM For 233 of these, aged 48 or over, information concerning the age at menopause was obtained from the case records or at interviews Five patients (2 per cent) had their menopause before age 43 and in all 40 (17 per cent) had it before age 48 [1]

Only few of the patients with early adult-onset DM, and none of the patients with juvenile DM had reached the age of 48 Data were obtained for nine such patients, four of these had their menopause before age 48

As is the case for menarche there is a time trend for menopause, but in the opposite direction the mean age at menopause seems to be gradually increasing Nevertheless the data may be interpreted to indicate a tendency that on average the menopause occurs somewhat earlier among diabetics than in the general population The material is not sufficiently large however, for a quantitative evaluation of this effect In addition it should not be overlooked that there can occur a selective element in so far as women with early menopause may be more prone to seek medical advice at a hospital than are women in general if they have DM (even in a mild form) it is very likely that the disease will be diagnosed in connection with their hospital visit

Pregnancies before the onset of DM

Of the 459 female patients in the Vänersborg series 448 are registered in the duration period 0–4 years (39 juvenile onset 73 early adult onset, 336 late onset) For these Table 45 shows the frequency of patients with previous pregnancy, the numbers of pregnancies, live births and overweight children (birth weight 4 500 grammes or over) and the mean number of live births per patient and per mother (woman with previous pregnancy), the data refer to the time of first admission and are given by age at onset

The total number of pregnancies is 808, and the total number of live births is 768 It can be concluded that there must be a certain underregistration in respect of the number of abortions (and stillbirths) For the 336 women with age at onset 40 or over, the average number of children (live births) was 2 1 per woman and 3 4 per mother no more than 62 per cent of these women had been pregnant However in this connection it should be taken into account that during the relevant years before the Second World War both the marriage rate and the birth rate were low in Sweden There was an increase of the marriage rate at the end of the thirties and in 1944–45 the birth rate was very high though this was connected more with what may be termed a displacement effect than with an increase of the total number of children per family during the

[1] According to recent statistics from the U S A (MacMahon & Worcester 1966) among women not having had operative menopause 29 per cent had natural menopause before age 48

Table 45 Pregnancies before first admission Duration 0–4, Vanersborg

Age at onset	Number of patients	With previous pregnancy		Number of			Mean number of live births per		Overweight children as percentage of live births
		Number	Per cent	Pregnancies	Live births	Overweight children	patient	mother	
0–14	39	–	–	–	–	–	–	–	–
15–24	31	2	6	4	4	–	0 1	2	–
25–29	13	7	54	7	6	2	0 5	1	33
30–34	18	10	56	20	20	5	1 1	2 0	25
35–39	11	7	64	19	19	–	1 7	2 7	–
40–44	16	11	69	29	29	7	1 8	2 6	24
45–49	36	21	58	63	61	4	1 7	2 9	7
50–54	52	35	67	112	107	17	2 1	3 1	16
55–59	65	45	69	156	145	11	2 2	3 2	8
60–64	60	43	72	171	158	14	2 6	3 7	9
65–69	46	19	41	72	68	11	1 5	3 2	16
70–74	36	21	58	91	88	2	2 4	4 2	2
75–	25	14	56	64	63	3	2 5	4 5	5
0–14	39	–	–	–	–	–	–	–	–
15–39	73	26	36	50	49	7	0 7	1 9	14
40–	336	209	62	758	719	[illegible]	2 1	3 4	10
Total	448	235	52	808	768	76	1 7	3 3	10

Overweight children birth weight 4 500 g or over

whole reproductive period For comparison it may be mentioned that in existing marriages in Sweden in 1935/36 contracted after 1900 the frequency of families with children was 92 per cent where the duration of marriage was 20–35 years and 90 per cent where the duration was 15–19 years, in marriages with children, the average number of children was 4 4 and 3 4 respectively (Sjostrand 1940)

Although there may exist selective elements in respect of the admission of DM patients to hospitals which are directly or indirectly connected with marital state and the number of children it is apparent that the data in Table 45 do not support a theory that the fertility of prospective DM patients is higher than the fertility in corresponding groups of the general population [1] This theory is not infrequently advanced in the literature, but in the main it seems to be based on a misinterpretation of the existing statistical data Usually these statistics refer to the number of sibs of DM patients however, it is not taken into account that the

[1] In the *Health Examination Survey 1960–62* the U S National Center for Health Statistics reports certain findings which may be interpreted as indicating that the average number of children is larger for diabetic women than for non-diabetic women (O Sullivan & Gordon 1966) Blood glucose levels were found to be unrelated to marital status and childbearing, but with increasing parity there was a rise in the (age adjusted) prevalence of diabetics According to the authors available data seem to favour the conclusion that pregnancy has no role in the causation or earlier appearance of diabetes The *Health Examination Survey* confirms the tendency toward higher parity among diabetic women without providing an explanation for it To attribute this increase in family size to an altered fertility rate or to some subtle psychosocial factors would be purely speculative

probability of at least one child in a family being registered as a DM patient is generally greater in a large family than in a small one (theoretically the only exception would be complete registration over a very long period)

If n_s is the number of families with s children and h is the whole life expectancy of a new born child being admitted to hospital because of DM the number h_s of families with s children in which at least one child will be admitted is

$$h_s = n_s[1 - (1 - h)^s]$$

or, where h is small,

$$h_s = n_s \; h \; s$$

Hence the average number of children in the families will be

$$\bar{s} = \frac{\sum s \; n_s}{\sum n_s} = \frac{\sum h_s}{\sum \frac{h_s}{s}}$$

$$\left[not \; \frac{\sum s \; h_s}{\sum h_s}\right]$$

The frequency of overweight children (4 500 grammes or over) does not show any clear tendency to vary with the age at onset of DM Nor did a scrutiny of the data reveal any clear variation with the family size For the whole series the frequency is 10 per cent

Pregnancies after the onset of DM

For the Vänersborg series the total number of children born to female DM patients after the first admission amounts to 45 Rearranging the data in Table 7B to show the distribution by average age (calculated from 5 year group by age at onset and 5 year group by duration) we find that in all there are 272 registrations (duration cards) which represent reproductive ages A survey of the data is given in Table 46, in which the registrations, the number of live births and the number of overweight children are shown according to the average age of the patients Further an approximate calculation of the birth rate by age has been made, for comparison, corresponding figures for the general population are shown (because of the great variation of the birth rates during the period these figures have been rounded off") In the evaluation of the table it should be observed that to a certain extent the fertility data for the DM patients are selective in so far as DM

Table 46 Pregnancies after first admission Vanersborg

Average age	Number of duration cards	Approximate number of observation years	Number of l e births	Number of overweight children	Approximate birth rate per thousand: DM patients	Approximate birth rate per thousand: General population 1950
19	37	145	–	–	–	65
24	52	220	7	–	32	130
29	52	220	14	–	64	120
34	47	200	19	3	85	90
39	46	170	4	2	24	40
44	38	160	1	–	6	10
Total	272	1 115	45	5		

might have been detected in connection with pregnancy and that this has then been the reason for admission, in particular this seems to apply to the group with average age 34 where 7 of the 19 children were born within the first duration period

It is evident from the table that the fertility of female DM patients is far below that of the general population

The frequency of overweight children (5 out of 45) agrees with that found among children born before the onset of DM (or, strictly speaking before the first admission)

Perinatal mortality

As already stated, there must be gaps in the registration of abortions The Vänersborg series covers a long period of time, during which there was a marked decline in early infant mortality (cf Larsson 1965) and considerable advances in the treatment of pregnant diabetic women The material from the investigation hospitals is not sufficiently large to permit a quantitative analysis of these developments

Pedersen (1954) investigated the perinatal mortality among children of diabetic mothers for the periods 1926–46 and 1947–52 His series comprises 230 mothers with 305 pregnancies and 309 children In 1947 Pedersen introduced a careful observance of pregnant diabetics with frequent controls of diet and insulin the treatment also comprised a rather long period of hospitalization before delivery in the 38th week rupture of membranes or caesarean section (12 cases) was performed Pedersen reports a decrease of mortality from 38 to 27 per cent for children weighing 2,500 grammes or less, and from 30 to 20 per cent for children weighing more than 2 500 grammes

A further decrease in perinatal mortality is reported by Pedersen (1965) viz 18 per cent for a series of 306 infants in 1959–63, and 14 per cent for 90 births in 1946–64, where the mothers were controlled 150 days or more before the calculated term Drury (1966) reports a perinatal mortality of 10 per cent

Problems related to pregnancy, delivery and mortality among diabetic mothers, and mortality among children of diabetic mothers are treated in a series of Swedish investigations, for instance those by Andersson (1950) Andersson and Swanberg (1961), Bergman (1953), N Bergqvist (1954), Brosset and Werko (1950), Hagbard (1956) Lunell (1966), Sundelin (1938), and Svanteson (1953)

The qualitative impression from our hospital series is in line with Pedersen s findings, but other factors, too, must have contributed to the improved outcome of pregnancies among diabetic mothers, for instance the availability of effective antibiotics and, not least, the steady amelioration of maternity and child welfare organization in Sweden

Acute complications

At any rate before the introduction in general therapeutic practice of sulpha preparations and antibiotics, diabetics were in general more often and more severely afflicted with infections than were non-diabetics The entries in the case records concerning infections are included

in the occasion cards, but not with the intention that these data should be processed separately although the greater part of the material belongs to a period when sulpha preparations and antibiotics were utilized an analysis of the data on infections would have only a limited historical interest However, we wanted to evaluate the occurrence of ketosis against the background of simultaneous infections

Acute infections

Data on the registered frequency of infections in each 5-year duration period (reckoned from the onset of DM) are included in Table 47 Of course it should be remembered that the registration mainly covers infections which have been found at the patient's visits to the hospital, the time intervals between these visits are in general shorter in the case of juvenile or early adult onset than in the case of late onset and they are in general shorter immediately after the first admission than they are later on

Diabetic coma and pre-coma

In respect of acidosis and ketosis we have refrained from processing the data on mild acidosis As is well known mild acidosis may often be a transient symptom, the significance of which is generally very difficult to evaluate The data which are given in Table 47, comprise solely instances where there has been ketosis to such a degree that an unquestionable disturbance of consciousness has occurred The less severe cases where the patient has been able to speak and to answer questions, albeit only by an effort are classified as pre-coma Where complete unconsciousness has been present the case is classified as diabetic coma

In the whole series there are registered 370 instances of pre-coma or coma For no fewer than 284 (77 per cent) of these the case records contain entries on infection it is generally agreed that infections are often the provoking factor for ketosis (cf Joslin Root, White & Marble 1959) These 370 instances of ketosis refer to 312 registrations (duration cards), several patients have in a 5 year duration period had more than one attack of pre-coma or coma A survey of the registrations is given in Table 47 by sex, age at onset (juvenile early adult late) and duration for a given duration period each patient is counted only once and the number of patients with registration of both ketosis and infection is related to the total number of patients in the duration period as well as to the number of patients with at least one registered infection during the period The first mentioned quotient will give a minimum expression for the occurrence of ketosis the last mentioned quotient, on the other hand may be assumed to give a fairly correct value for the frequency (in a 5-year duration period) of at least one instance of ketosis

As will be seen from the table ketosis (among patients with infection) has been registered for 2 per cent of the males and 4 per cent of the females with late onset DM for 8 and 12 per cent in the early-adult onset type and for 20 and 24 per cent, respectively in the juvenile-onset type For the juvenile diabetics the frequencies are higher, viz 31 and 32 per cent in the duration group 0–4 years For the early adult- and the late-onset types

Table 47 The occurrence of pre coma and coma by sex, age at onset and duration

Sex	Age at onset	Duration	Registrations (duration cards) Total T	With infection All I	With infection Per cent age I/T	Ketosis Pd Cd No	Ketosis Pd Cd Percentage of T	Ketosis Pd Cd Percentage of I	Occasions of ketosis No Pd Cd	Occasions of ketosis No Cd
M	0–14	0– 4	133	110	83	34	26	31	43	10
		5– 9	126	73	58	17	13	23	21	1
		10–14	121	60	50	3	2	5	3	1
		15–19	87	32	37	4	5	12	5	2
		20–24	48	22	46	2	4	9	2	–
		25–	33	9	27	–	–	–	–	–
		Total	548	306	56	60	11	20	74	14
	15–39	0– 4	386	244	63	22	6	9	23	6
		5– 9	252	110	44	11	4	10	12	2
		10–14	173	67	39	4	2	6	4	–
		15–19	124	47	38	2	2	4	2	–
		20–24	71	22	31	3	4	14	4	–
		25–	69	17	25	–	–	–	–	–
		Total	1 075	507	47	42	4	[illegible]	45	8
	40–	0– 4	944	499	53	10	1	2	12	7
		5– 9	469	185	39	2	0	1	2	–
		10–14	212	83	39	4	2	5	5	–
		15–19	101	34	34	1	1	3	1	–
		20–24	37	12	32	2	5	17	2	–
		25–	12	5	42	–	–	–	–	–
		Total	1 775	818	46	19	1	2	22	7
	All	Total	3,398	1 631	48	121	4	7	141	29
F	0–14	0– 4	125	119	95	38	30	32	48	8
		5– 9	115	86	75	22	19	26	31	8
		10–14	114	70	61	14	12	20	22	5
		15–19	68	45	66	9	13	20	9	3
		20–24	37	20	54	1	3	5	1	–
		25–	26	8	31	–	–	–	–	–
		Total	485	348	72	84	17	24	111	24
	15–39	0– 4	251	167	67	28	11	17	31	12
		5– 9	186	92	49	8	4	9	[illegible]	1
		10–14	131	64	49	7	5	11	8	1
		15–19	88	44	50	6	7	14	6	1
		20–24	58	21	36	1	2	5	1	–
		25–	58	25	43	1	2	4	1	–
		Total	772	413	54	51	7	12	56	13
	40–	0– 4	1 423	804	57	31	2	4	32	12
		5– 9	782	328	42	17	[illegible]	5	22	5
		10–14	340	149	44	5	1	2	3	–
		15–19	142	68	48	5	4	7	5	[illegible]
		20–24	33	16	48	–	–	–	–	–
		25–	10	5	50	–	–	–	–	–
		Total	2,730	1,370	50	56	[illegible]	4	62	19
	All	Total	3 987	2,131	53	191	5	9	229	58

Pd = diabetic pre-coma Cd = diabetic coma

no such difference is to be found in respect of pre-coma whereas the registered instances of coma mainly fall within the duration group 0–4 years (37 out of 49)

Hypoglycaemia, hypoglycaemic coma

When transferring the case record data to the occasion cards the occurrence of a blood sugar value below 0 06 per cent was classified as an instance of hypoglycaemia In addition the occurrence of hypoglycaemic coma was registered

During the later part of the investigation period hypoglycaemic coma (insulin coma) was seen only exceptionally on average one or two cases per annum were registered at each of the four departments of internal medicine The frequency may be somewhat higher at departments of paediatrics in particular during the adjustment of the insulin therapy

There are DM cases in which at the initial stages the production of insulin may be increased to such a degree as to result in hypoglycaemia However cases of this type are rare Otherwise, hypoglycaemia is regularly not a symptom (a sequela) of DM but a consequence of the treatment of the disease, in particular the administration of insulin Mild episodes of hypoglycaemia— insulin reactions — have occurred fairly often in the present series especially during the earlier part of the investigation period

As a rule the treatment of hypoglycaemia has been an adjustment of the administration of insulin, with a lower dose in the case of heavy muscular work or reduced food intake Occasionally, it has been found appropriate to give the patient a suitable quantity of sugar or other carbohydrates

Hypoglycaemic coma, with a blood sugar value of 0 04 per cent or less has regularly been treated by injection intravenously of glucose and glucagon or norepinephrine

During the first decades of insulin therapy, at least the consequences of hypoglycaemic states seem to have been underestimated Campbell, who worked together with Banting and Best when insulin was taken into therapeutic use appears to have been the first research worker to study the clinical problems connected with hypoglycaemia, his excellent paper on hypoglycaemia and hyperinsulinism (1958) is of great interest In recent years, the occurrence of certain cerebral changes after hypoglycaemia has been reported (Fahlgren Andersson & Lundmark 1957 Hartmann, Wohltmann Holowach & Caldwell 1960 Green 1963, Roberts 1964)

Tuberculosis

In order to detect instances of pulmonary tuberculosis and other types of tuberculosis there has been developed in Sweden a systematic activity at central and local dispensaries (about 60 and 650 in number) and X ray examinations on a large scale are undertaken (from 400 000 to 800 000 a year) The number of new findings of tuberculosis entered into the registers of central dispensaries has gradually decreased from about 3 1 per thousand inhabitants in 1943–44 to less than 0 5 per thousand in 1961–65 In 1961–63 the average annual number of new cases

was 3 769 (0 50 per thousand inhabitants) of which 2,604 (0 34 per thousand) were instances of pulmonary tuberculosis

The death rate for tuberculosis (A1–A5) as the primary cause of death was 0 81 per thousand in 1936–40, 0 69 in 1941–45 0 39 in 1946–50 0 16 in 1951–55 and 0 08 in 1956–60 the latest available statistics, for the year 1965 show a rate of 0 046 per thousand (males 0 063, females 0 029)

As already mentioned (p 103), the Central Bureau of Statistics has recently published certain data on causes of death in 1965, which include not only primary (underlying) causes but also complications and contributory causes As in respect of DM, tuberculosis (TB) does not appear as a complication but solely as the primary cause (TBp) or as a contributory cause (TBc) An extract of these statistics is shown in the following table (where as usual the annexed letter t stands for p + c)

1965		M	F	M + F
Average population thousands		3 862	3 872	7 734
Number of deaths T		42 031	36 163	78 194
Death rate per thousand		10 88	9 34	10 11
Number of deaths	TBp	242	113	355
	TBt	438	237	675
	Dp	565	769	1 334
	Dt	1 810	2 490	4 300
Death rate per thousand	TBp	0 06	0 03	0 05
	TBt	0 11	0 06	0 09
	Dp	0 15	0 20	0 17
	Dt	0 47	0 64	0 56
Death share per cent	TBp	0 58	0 31	0 45
	TBt	1 04	0 66	0 86
	Dp	1 34	2 13	1 71
	Dt	4 31	6 89	5 50

Among deaths in 1961–63 with DM as a contributory cause in all 2 940 males and 4,333 females there were 24 and 27 or 1 2 per cent and 0 6 per cent, respectively for which tuberculosis was stated as the primary cause From the 1965 table it can be seen that the total death shares for TBp are half of these values

As is apparent from these statistical data, the problems concerning the association of DM and TB have lost much of their interest in consequence of the gradual decline in the incidence and prevalence of TB, and the gradual increase in the overall prevalence of DM (due to reduced excess mortality among diabetics and changed age structure of the population) During earlier periods, a great many papers have been devoted to the study of these problems Root (1934) made a thorough review of the pertinent literature Of 1,121 diabetic autopsies 319 or 28 4 per cent revealed active tuberculosis, in a general series of 51,705 autopsies 22 9 per cent showed tuberculosis Taking into account that (for the periods studied) tuberculosis as well as diabetes entailed a comparatively heavy increase of the mortality risk Root concludes that active tuberculosis was found in diabetics at autopsy between two and three times as frequently as expected

In connection with the study by Silwer (1958) on the frequency of diabetics in Kristianstad County (L cf p 235) Oscarsson and Silwer (1958) investigated the prevalence of pulmonary tuberculosis among registered diabetics living on March 15, 1954 Of 1 326 diabetics, 1,270 were examined roentgenologically Pulmonary tuberculosis (not including clearly healed processes) was found for 46 or

3 6 per cent of these diabetics A comparison with the prevalence of pulmonary tuberculosis in the general population of the county (according to records at the dispensaries on December 31 1953) is shown in the following table

Attained age	Frequency of diabetics per cent	Number and frequency of pulmonary tuberculosis			
		Diabetics		Population	
		No	per cent	No	per cent
0– 9	0 06	–	0 0	50	0 1
10–19	0 17	2	3 5	101	0 3
20–29	0.22	[illegible]	8 5	359	1 0
30–39	0 21	4	5 3	580	1 6
40–49	0 38	9	7 1	459	1 3
50–59	0 85	5	2 1	365	1 3
60–69	1 59	9	2 5	216	0 9
70–	1 71	11	3 6	159	0 8
Total	0 51	46	3 6	2 289	0 9

After Oscarsson and Silwer (1958)

The frequency of pulmonary tuberculosis among diabetics aged 30 or over was about 3 5 times as high as the corresponding frequency in the general population The frequency of diabetes among patients with pulmonary tuberculosis was also about 3 5 times as high as the corresponding frequency in the general population (46 observed cases against 13 2 expected)

Oscarsson and Silwer conclude that diabetes especially when severe favours the development of tuberculosis the tendency is most obvious in patients who have had diabetes for a fairly long time Therefore all diabetics should be regularly examined by chest X ray so that any co-existing pulmonary tuberculosis may be discovered at an early stage

At the county hospitals included in our clinical study X ray control has been a routine procedure (as a rule once a year) There are comparatively few instances of tuberculosis registered in the series which is due, at least in part to the fact that the diagnosing of tuberculosis has preceded the onset of DM patients with active pulmonary tuberculosis are regularly treated at special hospitals (sanatoria) For this reason and because of the marked decline in the tuberculosis morbidity and mortality a statistical analysis of our series in respect of tuberculosis would be rather biased and of very limited value In the main it can be stated that nowadays tuberculosis is no real problem in the treatment of DM patients although such patients as well as other diseased persons should of course still be subjected to X ray control at suitable intervals

Chronic urinary-tract infections

Long standing urinary tract infections occur rather frequently among DM patients especially in females The diagnostic techniques in respect of these infections have been markedly improved during the last ten years and this has resulted in a rise in the number of registered cases Previously urine sediment was studied directly by microscopy and the results were evaluated mainly on the basis of the occurrence of leukocytes It was considered that infections of the urinary tract were 3–4 times as common in diabetics as in non diabetics

After the introduction of procedures in which quantitative analysis of bacterial culture from urine is utilized it has been possible to diagnose bacteriuria with greater accuracy In an unselected out patient series of 269 diabetics and 260 comparable non-diabetic controls, Vejls-

gaard (1965, 1965) found that 18 8 per cent of the diabetic females and 7 9 per cent of the non-diabetic females had bacteriuria (with more than 10^5 colonies/ml urine) For males the figures were very low (0 7 and 1 1 per cent, 1 and 3 instances of bacteriuria) Vejlsgaard concludes that it does not appear possible to correlate infection of the urinary tract with DM *per se* but that it seems likely that diabetic vascular disease is a contributory factor in the development of urinary infection in diabetic patients

As in respect of tuberculosis, a statistical analysis of our series would be of historical interest only, bacterial culture from urine has been made only in exceptional cases Reference may be made to the studies by Vejlsgaard (1965, 1965) Huvos & Rocha (1959), Isacsson (1965), Oseasohn, Liebow and Newill (1964) Parrish (1965), Silagy (1962), and Szucs Cserhati, Csapó and Balazs (1960)[1]

The occurrence of certain other diseases (thyrotoxicosis allergic diseases, malignant tumours rheumatoid arthritis, and pernicious anaemia) will be discussed in Chapter VII (pp 197–200)

[1] Reference may also be made to a study of renal complications and urinary tract infections among hospital patients with DM at the Turko University Central Hospital (Kasanen & Forsstrom 1964)

CHAPTER VI

Treatment

In the treatment of DM the main objective is to get the metabolic disturbance under proper control and to attain a metabolism comparable to that obtaining in healthy subjects Before the discovery of insulin and its introduction into general therapeutic practice, treatment was restricted to dietary procedures During that period—and long afterwards —DM was considered to be a disease consisting chiefly in a reduced capability of the body cells to metabolize carbohydrates Consequently it was only natural that the dietary prescriptions were aimed at cutting down the carbohydrate supply, the control of the disease was almost entirely directed to the carbohydrate metabolism The patient should not present urinary excretion of glucose and he should have a blood sugar level comparable to that of healthy persons To a great extent this objective has been maintained where insulin therapy is applied

Unfortunately the attainment of the goal thus set has proved far more difficult than was at first thought It has often been impossible to adapt the patient s working conditions to the diet in a way that would serve to keep a stable good control in respect of urinary glucose excretion and blood sugar content

Nowadays the great majority of clinicians agree that DM therapy should be designed to keep the patient s body weight at what is deemed to be normal and to secure the administration of such amounts of calories vitamins metals etc , as are sufficient for an adult patient to carry on his ordinary work and for a child to develop along lines as normal as possible Hence it is obvious that the therapy must be adapted to the needs and circumstances of the individual patients and must be suitably modified if substantial changes in these needs and circumstances should occur (cf Gronberg 1963) In the main these views agree with those expressed by Odin already in the 1930s (Odin 1939)

A penetrating discussion of the therapeutic problems would fall outside the scope of the present study Good surveys of these problems have been published by prominent diabetologists for instance in the volumes edited by Danowski (1964) Ellenberg and Rifkin (1962) Leibel and Wrenshall (1965) Oberdisse and Jahnke (1963) and Williams (1960) and in the classical textbook of Joslin Root, White and Marble (1959)

A thorough review of current conceptions concerning endocrine defects in DM and the metabolic effects of insulin is given by Renold and Cahill Jr (1966)

Our hospital series originates from a period when insulin exercise and diet

were the only general means applied in the treatment of DM, the use of oral remedies which is nowadays common, will be discussed in a later section

Diet therapy

On the basis of the entries in the case records the patient has in respect of each

Table 48 Diet by sex, hospital, age at onset and duration

Age at onset	Duration	Hospital	Males						Females					
			Number	Diet ( )					Number	Diet ( )				
				[illegible]	1	2	1	2		0	1	2	1	2
0–14	0– 4	P	47	4	70	26			39	10	57	33		
		G	33	6	85	9			28	7	[illegible]	7		
		W	23	4	87	9			31	6	88	6		
		Z	30	73	27	–			27	66	30	4		
		Total	133	20	67	13			125	21	65	14		
	5– 9	P	43	–	72	28	58	16	37	–	70	30	46	16
		G	32	3	[illegible]	9	75	9	29	–	97	3	62	3
		W	29	–	100	–	59	–	26	–	96	4	77	4
		Z	22	4	82	14	27	–	23	–	87	13	30	4
		Total	126	2	84	14	57	8	115	–	86	14	54	8
	10–14	P	40	–	82	18	58	8	32	3	66	31	44	16
		G	26	–	85	15	62	8	29	–	97	3	76	–
		W	30	–	100	–	70	–	32	3	97	–	59	–
		Z	25	8	[illegible]	4	28	–	21	10	85	5	33	–
		Total	121	2	[illegible]	10	55	4	114	4	85	11	54	4
	15–19	P	35	–	[illegible]	17	57	3	21	–	38	[illegible]	19	10
		G	17	–	94	6	41	–	14	–	100	–	79	–
		W	23	–	96	4	74	–	20	–	100	–	[illegible]	–
		Z	12	–	83	17	50	–	13	–	92	8	46	–
		Total	87	–	90	[illegible]	57	1	68	–	79	21	50	3
15–39	0– 4	P	115	10	37	53			73	10	38	52		
		G	66	3	74	23			42	7	86	7		
		W	115	3	95	2			82	5	91	4		
		Z	90	49	45	6			54	46	50	4		
		Total	386	16	[illegible]	21			251	16	66	18		
	5– 9	P	86	1	36	63	26	44	54	–	37	63	17	44
		G	47	2	70	28	51	17	33	3	85	12	42	3
		W	70	–	99	1	[illegible]	1	65	1	97	2	79	2
		Z	49	6	88	6	45	–	34	–	85	15	32	–
		Total	252	2	70	28	50	19	186	1	75	24	46	14
	10 14	P	59	5	37	58	22	36	35	–	34	66	17	46
		G	30	–	63	37	30	17	28	–	86	14	43	–
		W	51	4	92	4	69	–	45	–	[illegible]	2	73	[illegible]
		Z	33	–	100	–	39	–	23	–	87	13	35	4
		Total	173	3	70	27	40	15	131	–	76	24	45	14
	15 19	P	39	3	46	51	18	28	17	–	18	82	–	47
		G	28	–	89	11	29		20	–	80	20	35	5
		W	34		97	3	68	3	32	3	97	–	62	–
		Z	23	4	96	–	39	–	19	–	100	–	37	–
		Total	124	2	79	19	38	10	[illegible]	1	79	20	39	10

Continued

Table 48 Continued

Age at onset	Duration	Hospital	Males						Females					
			Number	Diet ( )					Number	Diet ( )				
				0	1	2	1*	2*		0	1	2	1	2
40–	0– 4	P	234	9	36	55			336	7	32	61		
		G	165	[illegible]	92	2			286	3	[illegible]	[illegible]		
		W	214	1	98	1			312	2	97	1		
		Z	331	40	58	2			489	34	61	5		
		Total	944	17	68	15			1 423	15	67	18		
	5– 9	P	129	4	25	71	17	53	191	–	13	87	8	59
		G	79	–	94	6	81	4	175	1	[illegible]	10	67	7
		W	113	–	98	2	69	1	201	–	99	1	71	1
		Z	148	3	93	4	45	–	215	8	83	9	46	2
		Total	469	2	76	22	49	15	782	[illegible]	71	26	48	17
	10–14	P	64	–	31	69	11	45	76	1	8	91	4	57
		G	32	–	88	12	69	[illegible]	83	–	[illegible]	16	49	6
		W	58	–	100	–	66	–	96	1	98	1	71	–
		Z	58	2	88	10	45	3	85	2	87	11	44	2
		Total	212	0	75	25	44	16	340	1	72	27	44	15
	15–19	P	35	–	34	66	11	34	26	–	27	73	4	31
		G	14	–	79	21	36	7	36	–	86	14	36	8
		W	29	–	100	–	72	–	46	–	96	4	59	2
		Z	23	4	96	–	52	–	34	–	85	15	50	–
		Total	101	1	73	26	42	13	142	–	78	22	41	8

Diet in the duration period 0 = entirely free
1 = partially regulated
2 = totally regulated

* = unchanged code since first admission (there are only 13 patients 0*)

admission been classified under one of the following five headings which so far as possible refer to the actual diet (not to the prescribed diet)

(a) entirely free diet (including sugar, sweets, etc)

(b) free diet with limitation of sugar and sugar containing products but without other restrictions

(c) normal diet without sugar and sweets

(d) partially regulated diet free from sugar and sweets

(e) totally regulated diet with fixed daily amounts of carbohydrates lipids and proteins

It was thought that in this way it might be possible to arrive at an at least approximate idea of the types of food the DM patients were taking Quite naturally, the borderlines are not always clear cut this proved to apply in particular to the divisions between groups (b) (c) and (d) When processing the occasion cards into duration cards we therefore decided to use three classes only, viz

(0) entirely free diet (a)

(1) partially regulated diet (b, c d)

(2) totally regulated diet (e)

The data for the durations 0–4 5–9 10–14 and 15–19 years are given in Table

Table 49 Insulin treated patients by sex, hospital age at onset and duration

Duration	Hospital	Juvenile onset (0 14) Number M	F	Insulin ( ) M	F	Early adult onset (15 39) Number M	F	Insulin ( ) M	F	Late onset (40) Number M	F	Insulin (%) M	F
0– 4	P	47	39	96	97	115	73	92	89	234	336	57	56
	G	33	28	100	100	66	42	98	95	165	286	84	74
	W	23	31	91	97	115	82	85	89	214	312	62	53
	Z	30	27	100	100	90	54	88	89	331	489	60	58
	Total	133	125	97	98	386	251	91	90	944	1 423	64	60
5– 9	P	43	37	100	100	86	54	95	94	129	191	81	78
	G	32	29	100	100	47	33	100	97	79	175	92	78
	W	29	26	100	100	70	65	97	97	113	201	70	62
	Z	22	23	*100*	*100*	*49*	*34*	*94*	*100*	*148*	*215*	*80*	81
	Total	126	115	100	100	252	186	96	97	469	782	80	75
10–14	P	40	32	100	100	59	35	98	100	64	76	80	91
	G	26	29	100	100	30	28	100	96	32	83	88	80
	W	30	32	100	100	51	45	96	93	58	96	91	81
	Z	25	21	100	100	33	23	100	100	58	83	83	89
	Total	121	114	100	100	173	131	98	97	212	340	85	85
15–	P	67	42	99	100	90	46	99	85	53	41	72	83
	G	29	26	*100*	*100*	*54*	*45*	*98*	*100*	*24*	*44*	*83*	*95*
	W	47	47	96	100	79	79	82	89	40	58	97	81
	Z	25	16	100	100	41	34	93	100	33	42	85	98
	Total	168	131	98	100	264	204	93	92	150	185	83	89

48 by sex, hospital and age at onset (juvenile early adult late) In addition to the number of patients (duration cards) there are shown the percentage distribution by type of diet during the period in question and further the percentages of patients who have remained on diet (1) or (2) since their first admission

In the first duration period (0–4 years) entirely free diet was practised for 15–20 per cent of the patients At Vänersborg (P) Växjo (G) and Falun (W) the figures are below 10 per cent whilst at Östersund (Z) they are much higher especially for juvenile diabetics In the following duration periods (5 years or more) entirely free *diet was used only exceptionally For juvenile diabetics totally regulated diet* was applied to rather more than a quarter of the patients at Vänersborg and to less than 10 per cent of the patients at the other three hospitals For diabetics with early adult or late onset, totally regulated diet was used at Vänersborg for more than half of the patients, at the other three hospitals totally regulated diet was applied to a comparatively small extent

In the duration group 10–14 years about 60 per cent of the patients under observation carried on with the diet introduced after the first admission However it should be remembered that in this respect the material may be biased in so far as patients who are reluctant to follow the doctor s prescriptions may be more prone to vanish than are patients who are willing and able to cooperate

Insulin therapy

By sex hospital age at onset and duration Table 49 shows the frequency of patients treated with insulin Roughly speaking insulin therapy was applied to all patients with juvenile onset DM to about 90 per cent of the patients with early adult onset DM, and to more than 60 per cent of the patients with late-onset DM Insulin therapy was used more frequently at Växjo (G) than at the three other hospitals It can further be seen from the table that for patients with late onset DM the frequency figures increase markedly with increasing duration Very likely this is an expression of certain selective factors it is conceivable that many patients who are able to manage their DM by means of diet and exercise alone will prefer to obtain the necessary control examinations outside the county hospital if they have easy access to a doctor or a local hospital in or near their place of residence In addition it is likely that the increase with time of the number of first admissions has brought about an 'admixture of comparatively mild cases in the first duration group (0–4 years) Finally, it should be noticed that the tabulation relates to the use of insulin during each 5 year registration period There are many instances where a patient was kept on diet for one or two years and then was prescribed insulin, if insulin was taken at all during the period the patient is counted as 'with insulin

As already mentioned the oldest part of the present series originates from a time when only regular insulin was avail able but since about 1935 it has been possible to use long acting insulin

Both types were widely used At first, it was considered that the two types should be injected separately hence, if a patient had insulin with two doses a day three or four injections were needed However, it turned out that many patients—with or without the doctor s permission—mixed the two preparations and took the injections twice a day The consequence of this was that the treatment came to comprise more long acting insulin and less rapid acting insulin, protamine zinc insulin contains an excess of protamine and zinc which binds regular insulin Thus there could be no certainty about the duration time of the mixture but nevertheless the results were as a rule satisfactory

With regard to the objective of the treatment the views among clinicians still differ There are physicians who are of opinion that—by means of a strict diet and an ample supply of insulin—the DM patients should be kept at a normal blood sugar level and be free from sugar in the urine Others consider that no more insulin should be administered than is necessary to keep the urinary excretion of glucose below 30 grammes per day and that the blood sugar level should not be reduced so much that hypoglycaemia may occur There seem to be special arguments in favour of this latter view among other things, recent observations have shown that hypoglycaemia may cause cerebral damage (cf p 145)

It is rather difficult to describe briefly the techniques used during the investigation period for the administration of insulin It might be said that each patient had his individual way of taking the insulin and that each of the doctors had

his own views concerning how and when the insulin injections should be given With some generalization the following pattern can be said to emerge

(a) *Juvenile onset*

Dietary regulation alone was rare Generally the onset of the disease is acute and accompanied by considerable ketosis, and therefore even a dietary treatment of short duration may be dangerous As a rule treatment with regular insulin in two or more doses daily was introduced without delay during the earlier part of the investigation period rather large doses were often given Usually proper control was rapidly established and as soon as the acidosis had been eliminated attempts were made to arrive at an adequate adjustment of the insulin treatment—sometimes a delicate problem Often the patient and his family were anxious to have the insulin injections reduced as much as possible and preferably discontinued The doctor having reasons for trying to keep good control preferred that injections should be given at least twice a day Even where the doctor felt that one injection with long acting insulin would not be wholly appropriate he had often to compromise in order to secure that at least some insulin treatment would be continued Quite often those patients who where given their insulin once a day got on surprisingly well in spite of large urinary excretion of sugar (and large quantities of urine) it is a strange and still unexplained phenomenon that many young DM patients can tolerate without any great discomfort a condition of high blood sugar large quantities of urine and large urinary excretion of sugar However, it has frequently been seen in the present series that trouble occurred when the patients attained the age of 15 or 16 and then an adjustment to two injections a day possibly with a mixture of regular and long acting insulin, proved essential—a change that sometimes necessitated intense and tiresome discussions

(b) *Early adult onset*

As already pointed out this category (with age at onset 15–39) should be regarded as an intermediate group Some of the patients presented typical juvenile DM Others especially those who fell ill at ages 25–39, displayed a clinical picture similar to the DM of old age

In respect of those patients who had DM of the juvenile type the treatment was on the whole similar to that described in connection with patients with onset below age 15 For the other patients it was characteristic that the onset of DM was less acute (although not really insidious) that the occurrence of the disease was often revealed incidentally and—above all—that the patients were acidosis resistant and far more stable in respect of blood sugar level and other symptoms than were juvenile diabetics Of these patients the great majority were treated initially by dietary regulation with the dual purpose of arriving at conclusive data on carbohydrate balance etc and convincing the patient that insulin treatment where deemed appropriate was not introduced unnecessarily Sometimes the dietary regulation was sufficient to keep the patient under good control for many years When insulin had to be used recourse was generally had to

long acting insulin with one daily injection Quite often this worked excellently However many patients could not be kept under good control in this way and in these cases the treatment was regularly adjusted to comprise two injections a day —ordinarily with a mixture of regular and long acting insulin, in particular this mixture technique was applied after the introduction of N P H insulin (non protein Hagedorn) which can be given together with regular insulin without any disadvantages

(c) *Late onset*

As a rule the onset of DM is insidious or at any rate is less alarming than among juvenile diabetics, there is no tendency to acidosis Often the endogenous production of insulin is preserved, at least during the first years after the onset of the disease Not seldom the occurrence of DM was revealed in connection with intercurrent disease

As a rule the treatment started with dietary regulation along the lines described under (b) However as will be seen from Table 49 there were a great many patients with late onset DM for whom insulin treatment was considered warranted the criteria applied at Växjo (G) in this respect seem to have been different from those applied at the three other hospitals

In this group it often proved adequate to keep the treatment at one daily injection of long acting insulin It was a common experience that the administration of insulin could without disadvantage be restricted to a comparatively small dose An additional motive for this choice was that for social reasons it is often desirable to make the insulin treatment as easy as possible, many of the patients were old and in need of help with the injections, and of course it is more convenient for a district nurse or a family member to give one injection daily instead of two Here, too it was often a question of compromises

As will have appeared from this description the insulin treatment registered in our hospital material cannot be regarded as an expression of a scientifically founded system but rather as representing a series of practical considerations regarding which one may say that they functioned much better than one could justifiably have expected

Type of insulin

By sex age at onset and duration, Table 50 shows the percentage distribution according to the type of insulin given The table refers to patients treated with insulin during each registration period and a grouping is made into three categories viz

(1)=regular insulin alone

(2)=both regular and long acting insulin either with a change over the period or with parallel administration (with separate injections or more commonly with injection of mixture)

(3)=long acting insulin alone

Number of injections

For patients who had been prescribed insulin in a duration period Table 51

Table 50 Type of insulin by sex age at onset and duration

Age at onset	Duration	Males treated with insulin: Number	Type of insulin ( ): 1	2	3	1*	2	3	Females treated with insulin: Number	Type of insulin ( ): 1	2	3	1*	2*	3
0-14	0- 4	129	30	61	9				123	38	55	7			
	5- 9	126	29	51	20	15	35	4	115	22	65	13	14	39	2
	10-14	121	22	56	22	11	22	3	114	18	67	15	9	26	3
	15-19	85	21	60	19	13	12	2	68	18	73	9	6	22	1
	20-24	48	27	58	15	17	10	–	37	19	76	5	11	8	–
	25-	32	41	28	31	16	6	6	26	31	54	15	4	19	–
	Total	541	27	55	18				483	24	65	11			
15-39	0- 4	351	23	60	17				226	26	60	14			
	5- 9	243	26	53	21	17	39	5	180	22	59	19	16	38	3
	10-14	170	26	54	20	15	26	3	127	14	71	15	10	29	2
	15-19	121	27	51	22	14	17	1	86	17	62	21	10	19	–
	20-24	68	26	50	24	15	6	1	54	17	55	28	9	11	2
	25-	56	41	41	18	16	2	4	48	21	54	25	8	10	2
	Total	1 009	26	54	20				721	21	61	18			
40-	0- 4	60-	16	48	35				848	15	49	36			
	5- 9	376	16	43	41	8	26	19	584	13	47	40	5	25	17
	10-14	180	16	42	42	6	16	17	289	17	46	37	6	19	9
	15-19	89	17	41	42	6	4	12	127	13	44	43	2	13	9
	20-24	29	10	42	38	3	14	–	29	10	52	38	3	17	–
	25	7	29	57	14	14	–	–	8	25	75	–	12	–	–
	Total	1,285	16	46	38				1 885	15	48	37			

Type of insulin used during the period 1 = regular
2 = mixed or with change during the period
3 = long acting

* = unchanged code since first admission to the hospital

gives the *percentage distribution according* to the number of insulin injections per day It should be noticed that the data are averages of the number of injections stated *in* respect of admissions during the registration period in question admissions resulting in dietary regulation alone are included (*i e they are not* excluded from the denominator) Further it should be noticed that the data refer to registered prescriptions (or corrected registration data on prescriptions) the patients may have applied a different frequency of injections without telling the doctor and it is of course impossible to correct the data for deviations of this kind On the whole it seems likely however that the patients took care to follow the prescriptions [1]

The data in Table 51 are given by sex hospital, age at onset (juvenile, early adult late) and 5 year duration periods (0–19 years) [2]

As will be seen from Table 51 there are considerable differences between the four

[1] It is quite another matter that a number of patients who did not come back to the hospital might have sought advice from other doctors or hospitals, in order to obtain an "easier" treatment.

[2] There are in all 17 registrations for which data on the number of injections during the period are lacking; for convenience these "gaps" have been distributed proportionately within their respective group by sex hospital age at onset and duration

hospitals Looking at the first duration period (0–4 years) we find that as regards juvenile-onset DM one injection per day was applied at Östersund (Z) for more than half of the patients, the corresponding figures at the three other hospitals vary around 20 per cent In respect of early adult-onset DM one injection per day was applied for nearly three quarters of the patients at Östersund (Z) and for about two thirds of the male patients and half of the female patients at Falun (W) but only for one third of the patients at Växjö (G) and one fifth of the patients at Vänersborg (P) As regards late-onset DM one injection daily was applied for about 90 per cent of the patients at Östersund and about 80 per cent of the patients at Falun and Växjö but for no more than about 40 per cent of the males and 60 per cent of the females at Vänersborg In part these differences are a reflection of geographical and demographic dissimilarities between the admission areas of the

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Quantity of insulin

There are no laboratory methods available which make it possible to measure by routine procedures the requirements of daily insulin supply to a DM patient. By means of rather complicated investigations it is however possible to determine whether or not a patient has a preserved capacity of producing insulin

During the first few years after the introduction of insulin into therapeutic practice it was considered that the amount of insulin needed was proportional to the quantity of carbohydrates administered one I U (international unit) of insulin was regarded as capable of bringing about the combustion of 4 grammes of carbohydrates This rule of thumb however has not proved tenable

The decision concerning the insulin quantity to be given to a patient was generally based on a number of clinical observations regarding blood sugar level, urinary excretion of glucose presence or absence of acidosis quantity of urine body weight and—not least—the patient s own statements as to his condition and symptoms In the present series adjust-

the insulin treatment should be made fairly soon after the first discharge On the other hand it is evident that a great

ERRATA

table 2	For explanation asterisks see page 42 in te
table 5 6 7 32	CO_2 not in mEq/L but in mM/L
table 37	* protein concentrations in g/Kg H_2O ** number of samples from which correlation between water content and protein concentration was calculated

many patients were kept at the dose of insulin that was settled during their first hospital stay

Of course to a certain extent concurrent complications such as infections, operations, etc, led to a temporary ad

Table 51 Number of insulin injections per day by sex hospital, age at onset and duration

Age at onset	Duration	Hospital	Males treated with insulin				Females treated with insulin			
			Number	Injections per day ( )			Number	Injections per day ( )		
				1	2	3		1	2	3
0–14	0–4	P	45	20	67	13	38	8	79	13
		G	33	15	76	9	28	[illegible]	64	18
		W	21	29	71	–	30	17	[illegible]	–
		Z	30	56	37	7	27	52	28	20
		Total	129	29	62	9	123	22	[illegible]	12
	5–9	P	43	2	82	16	37	3	73	24
		G	32	28	53	19	29	14	52	34
		W	29	21	76	3	26	15	77	8
		Z	22	68	23	9	23	56	35	9
		Total	126	25	62	13	115	19	61	20
	10–14	P	40	5	90	5	32	3	94	3
		G	26	31	57	12	29	21	65	14
		W	30	27	73	–	32	28	72	–
		Z	25	40	56	4	21	52	43	5
		Total	121	23	72	5	114	24	71	5
	15–19	P	34	15	79	6	21	5	95	–
		G	17	12	76	12	14	21	79	–
		W	22	32	68	–	20	40	60	–
		Z	12	67	33	–	13	54	31	15
		Total	85	26	69	5	68	28	69	3
15–39	0–4	P	106	23	72	5	65	15	[illegible]	3
		G	65	33	51	16	40	30	60	10
		W	101	65	33	2	73	53	46	1
		Z	79	72	27	1	48	72	26	2
		Total	351	48	47	5	226	42	54	4
	5–9	P	82	11	82	7	51	2	[illegible]	14
		G	47	23	68	9	32	31	53	16
		W	68	66	33	1	63	63	37	–
		Z	46	60	38	2	34	44	41	15
		Total	243	38	57	5	180	37	54	9
	10–14	P	58	2	91	7	35	3	97	–
		G	30	20	67	13	27	30	66	4
		W	49	51	47	2	42	55	43	2
		Z	33	64	36	–	23	[illegible]	35	4
		Total	170	31	64	5	127	36	62	2
	15–19	[illegible]	38	3	83	14	17	6	82	12
		G	27	22	74	4	20	75	70	5
		W	33	39	61	–	30	60	40	–
		Z	23	61	39	–	19	79	16	5
		Total	121	28	67	5	[illegible]	45	50	5

Continued

Table 51 : Continued

Age at onset	Duration	Hospital	Males treated with insulin: Number	Injections per day ( ) 1	2	3	Females treated with insulin: Number	Injections per day ( ) 1	2	3
40–	0– 4	P	133	41	59	–	188	59	39	2
		G	138	80	20	–	213	81	19	–
		W	133	83	16	1	163	78	20	2
		Z	200	91	9	–	284	[illegible]	12	1
		Total	604	76	24	0	848	78	21	1
	5– 9	P	105	29	71	–	149	42	57	1
		G	73	80	19	1	136	74	25	1
		W	79	86	14	–	125	78	21	1
		Z	119	86	14	–	174	87	11	2
		Total	376	69	31	0	584	70	29	1
	10–14	P	51	22	78	–	69	25	75	–
		G	28	64	36	–	66	70	25	5
		W	53	75	25	–	78	69	31	–
		Z	48	85	13	2	76	77	23	–
		Total	180	61	38	1	289	60	39	1
	15–19	P	28	25	71	4	22	23	72	5
		G	12	50	42	8	35	54	46	–
		W	28	89	11	–	37	70	30	–
		Z	21	75	25	–	33	75	25	–
		Total	89	60	38	2	127	59	40	1

Number of injections per day (average for all admissions during the registration period): 1 = one or less; 2 = more than one but not more than two; 3 = more than two

justment of the insulin dose As far as possible these intermediary doses have been excluded from the data given below concerning the average daily quantity of insulin

It is considered that a healthy person has a daily endogenous insulin production of 40–60 I U Therefore we have chosen to classify the registered administration of insulin into the following three categories

(1) 40 I U or less
(2) 41–80 I U
(3) more than 80 I U

By sex hospital age at onset (juvenile, early adult late) and 5 year duration groups (duration 0–19 years) Table 52 shows the percentage distribution of the patients who during the duration period in question took insulin according to the average daily quantity of insulin prescribed at each admission It should be noticed that the averages relate to *all* admissions (occasion cards) in the period and hence even those admissions where no insulin treatment was prescribed Patients without insulin during all admissions (viz average value 0) are not included the table deals solely with patients who took insulin during the duration period As already mentioned, intermediary doses have as far as possible been excluded from the calculation of the averages

As will be seen from the table, there is

(G) and Falun (W) occupy an intermediate position At Växjo the amounts are comparatively large for early adult onset DM but comparatively small for late onset DM whereas the opposite can be said to apply in respect of Falun

Complications and preventive measures

Data on the occurrence of glycosuria ('control) have already been given in Chapter V (Tables 37 and 38) and the observations with regard to overweight and blood pressure have also been discussed there (Tables 40 and 41 Tables 43 and 44)

Acute complications

Data on the occurrence of ketosis diabetic pre coma and diabetic coma during different duration periods are given in Chapter V (Table 47), and only a few comments will be added here

The generally accepted view is that ketosis is due to a disturbance of the fat metabolism Triglycerides and fatty acids do not undergo complete combustion the oxidation stops at too early a stage and in consequence a poisoning with betahydroxybutyrate acetoacetate and acetone occurs

Before (and immediately after) the introduction of insulin therapy diabetic coma was a common cause of death among DM patients nowadays however fewer than 1 per cent of the diabetics die from diabetic coma

Before the introduction into general therapeutic practice of insulin sulpha preparation and antibiotics acute infections—in particular streptococcic infections—were serious complications, nowadays they play a very small role since they can be kept under control by means of available remedies

Late complications

As already mentioned in Chapter I the occurrence of the so-called late complications of DM—diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy—has attracted much attention during the last few decades It is considered that retinopathy and nephropathy, and in part neuropathy are due to or connected with specific capillary changes—diabetic microangiopathy Knowledge of the cause and origin of these changes is still rather limited but it has been established that microangiopathy is often present already at the time when it is possible to make the clinical diagnosis DM (cf for instance Rifkin & Leiter 1962)

There have been divergent opinions concerning the proper prophylactic and therapeutic measures to be taken with regard to the late complications A central topic in the discussions has been the role played by the diet Definite proofs that a strictly regulated diet prevents the late complications or makes their course milder do not seem to have been given and in fact it must be extremely difficult to arrive at such proofs A less debated question has been whether the application of different types of insulin therapy might entail different consequences for the development and course of late complications

Vascular diseases constitute an important problem in clinical diabetes re

search It has long been considered that to a great extent DM patients are prone to develop vascular diseases especially cardiosclerosis with cardiac infarctions For this reason attention has been directed to the role of lipids in the occurrence of vessel damage Through thorough studies Kinsell and co workers (1965) have shown that the blood cholesterol can be kept at a low level by means of a suitable diet with small amounts of fat preferably in the form of polyunsaturated fatty acids Yet it seems to be an open question whether or not this level is of the same significance for all DM patients It may be taken as certain that a reduction should be aimed at so far as DM patients with pathologically increased blood cholesterol are concerned Possibly it is not equally certain that a depression below normal level will be beneficial

Recent investigations for instance by Östman (1965) have revealed that the lipid tissues consume comparatively large quantities of insulin These findings are additional arguments for the prescription of a diet poor in fat and for a general reduction of fat deposits i e a comparatively low body weight

With the present state of knowledge it has not been possible to arrive at unambiguous conclusions concerning the aetiology of the late complications of DM or even concerning the effects of therapeutic measures on the course of these complications DM is a disease where much attention must be given to each individual patient and it would not be advisable to perform prospective studies over long periods involving differential treatment of strictly comparable patients Retrospective studies of patients who have been treated differently—for instance at different hospitals or at the same hospital during different periods of time—are as a rule difficult to evaluate that a number of selective factors occur (or at least may be suspected to occur) has been shown in the previous chapters The quantitative data from our series in respect of late complications will be presented and discussed in Chapter VII

Working capacity

On the whole it might be stated that the working capacity of the majority of DM patients does not differ from that of comparable persons in the general population For DM patients of working age the diet and the administration of insulin do not in ordinary situations interfere with their working capacity With the availability of sulpha preparations and antibiotics the absence from work in the case of infections need not be longer for diabetics than for non-diabetics Of course stay in hospital for adjustment of the treatment and for training will imply an extra loss of working days but on the other hand this will at least to a great extent be compensated by the necessity for many diabetics to lead a very regular life and—often—their demand for physical activity

The real obstacle to the working capacity of diabetics is the loss of visual acuity which may be caused through retinopathy From the socio-economic point of view it can be said that the possible reduc-

tion of vision is decisive for the appraisal of the working capacity of a diabetic With the introduction in Sweden of a general system for invalidity survivors and old age pensions the reluctance—or even in certain quarters, refusal—of employers to engage diabetics has largely disappeared although a few traces of the earlier views still remain Factors contributing to the change for the better have been the establishment of effective organizations for occupational guidance and for the retraining of partially incapacitated persons and the general situation on the labour market which has been characterized by high employment and manpower shortage during the whole postwar period Before the Second World War (and even for some time afterwards) there was a much greater disinclination than now to employ diabetics in positions carrying pension rights

Other late complications may of course cause in severe cases a considerable decrease or even a total loss of working capacity but then the disease will in general have progressed so far that the remaining expectation of life is much reduced

The crucial complication in respect of length of life is nephropathy as long as the kidney functions are not too greatly disturbed the diabetic is ordinarily able to carry on his work and when renal insufficiency has developed the patient will in general have only a short time left

Neuropathy has not proved to entail any appreciable loss of working capacity mainly because of the peculiar circumstance that pareses of importance do not occur As a rule diabetics with neuropathy are able to walk and move unconstrainedly, in spite of an often considerably reduced sensation in feet and legs Generally there is a degeneration which disturbs deep sensation and sensitivity to pain, whereas sensitivity to contact is comparatively well preserved

With regard to the type of work, it is interesting to note that a great many of the DM patients in our series were anxious to be able to keep on with an occupation involving heavy muscular exertion

Oral hypoglycaemic agents

As early as 1914, long before the discovery of insulin, it had been found in experiments that an animal on which parathyroidectomy had been performed not only developed tetany as a consequence of disturbed extracellular calcium balance but also presented hypoglycaemia Watanabe (1918) associated the latter phenomenon with the occurrence of an increased amount of guanidine in the blood and this became the starting point for attempts to treat DM with guanidine preparations During the thirties trials were made (Synthalin) but it was not until 1958 that such preparations—in particular phenylethylbiguanide (Dibein Phenformin DBI)—were at all frequently brought into use they have been applied at the investigation hospitals in addition to insulin in difficult cases and have been well tolerated by the patients (cf Evaldsson & Grönberg 1961)

In 1946 Loubatieres observed that patients who were treated with sulphonamides because of infections sometimes displayed drowsiness which was shown to be due to hypoglycaemia Loubatieres

(1946) was able to prove that the reduction in blood sugar level was connected with the patient s production of insulin, when pancreatectomy was performed on experimental animals no similar effect was to be seen On the basis of these observations a number of sulphonylurea preparations has been produced and brought into use for stimulating the β cells of the Langerhans islands to produce more insulin (cf Creutzfeldt & Soling 1961) At the investigation hospitals several of these (Carbutamide or BZ 55, Tolbutamide or D860 Chlorpropamide or Diabinese) have been applied in particular to replace or postpone the adminis tration of insulin in cases of late onset DM Investigations have shown that the frequency of complications is approxi mately the same for all these different sulphonylureas (Östman & Gronberg 1964)

Recently a sulphapyrimidine prepara tion has been introduced (Lycanol or Gondafon) Its effects seem to be comparable to those of the sulphonylureas (Gronberg Krook & Sjoholm 1967)

As already mentioned, oral hypo glycaemic agents were used to only a small extent at the investigation hospitals during the period covered by our clinical series From the last years of the 1950s up to the present time the following treat ment procedures have been practised

(a) Biguanides have been used to supplement insulin treatment of juvenile and early adult onset DM especially where it has proved difficult to arrive at satisfactory control or where it has been desirable to restrict the injections to one a day

(b) Patients with late-onset DM treated with diet only, are nowadays often prescribed appropriate doses of sulphonylurea If this does not prove to give satisfactory control biguanide is added and by means of this the resort to insulin can usually be avoided or at least postponed for years

(c) Where patients who have been given sulphonylurea (with or without the combination with biguanide) prove to need insulin the oral remedies are regularly withdrawn, and the treatment is then diet and insulin alone In certain cases an additional dose of biguanide can be prescribed after some time on the indications mentioned above under (a)

(d) In the case of patients with marked overweight, biguanide is sometimes given as the only remedy

The general statement can be made with regard to the oral agents that they are valuable instruments for the treatment of DM in certain situations, but they cannot be used as real *substitutes* for insulin

Smedby (1966) investigated a sample of the Swedish population (persons born on February 15) with regard to prescriptions paid for wholly or partly by the national health insurance (cf [illegible] 19) in 1963 There were registered 206 DM patients who had taken out insulin and oral hypoglycaemic agents with the following distribution

Age	Insulin alone	Insulin plus oral	Oral alone	Total
–44	41	5	3	49
45–64	24	8	34	66
65–	28	3	60	91
Total	93	16	97	206

In a study of the greater part of Norrbotten County (BD) Meyer Lie and Smedby (1967) arrived at the following results in respect of medicine provided free of charge in 1963

Age	Insulin or insulin + oral	Oral alone	Total
0–14	46	3	49
15–44	300	27	327
45–64	275	396	671
65–74	158	338	496
75–	77	185	262
Total	856	949	1 805

Approximately the number of persons receiving DM medicines free of charge amounted to 0 75 per cent of the population At ages 65 and over the prevalence figures were about 2 7 per cent for males and 4 5 per cent for females

It should be borne in mind that a great many elderly diabetics are treated by means of dietary prescriptions and do not receive either insulin or oral hypoglycaemic agents and hence the prevalence of DM must be higher than indicated by these percentages

The incidence of persons receiving free DM medicines for the first time in 1963 was about 1 1 per cent In the age group 65–74 the incidence figures were 4 6 per cent for males and 6 0 per cent for females and at ages 75 and over they were still higher, viz 5 1 per cent for males and 9 8 per cent for females

In the evaluation of these latter figures it should be borne in mind that in respect of patients receiving DM medicines free of charge for the first time it is likely that there is in Norrbotten County an acceleration effect (as has probably been the case in most other counties in the past ten years)

CHAPTER VII

Late complications

Basically the clinical part of this study is a *retrospective* investigation of *actual* registrations made over a long period at four carefully selected county hospitals in different parts of Sweden During the period studied the physicians in chief at the departments of internal medicine in all these hospitals have taken great interest in the treatment of DM patients

In our opinion the results obtained from the analysis of the data on late complications are highly illuminating in so far as they prove the necessity of the utmost caution in the use of hospital register data (even very extensive case records) for conclusions concerning geographical differences and changes with time of the incidence and prevalence of late complications of DM (and of other similar hospital data) Intentionally the presentation in this chapter will be devised to illustrate the difficulties obtaining in these respects, this part of the analysis will mainly be made by means of *standard comparisons*

Diabetic retinopathy (Rd)

The first known description of a case of retinopathy was published in 1855 by Jaeger (cf Fischer 1957) As the grave cases of Rd mostly appear after a diabetes duration of one or two decades it was not until insulin treatment had been in use for many years that the importance of Rd as a complication of DM was generally known Since then Rd has been the subject of extensive research dealing with the symptoms treatment and prognosis of Rd and studying the factors which may affect its development and course (cf for instance Caird and Garrett 1963, Granstrom 1947 Gronberg and Svanteson 1951 Nordlow Gronberg and Svanteson 1953 Lundbæk 1955, Mårtensson and Palm 1950, Deckert 1960 Engel 1950 Johnsson 1959 and 1960 Jokipii 1953, Jorde 1961)

Reports on the general frequency of Rd differ widely The diagnosis of Rd depends on the definition used, the available technical resources and the knowledge and competence of the investigator As the development of Rd is correlated strongly to the duration of DM and weakly to the patient s age the older reports where these factors are not separated are often not comparable, owing to bias in the selection of patients A rough survey of research results during the last four decades is given in Table 53 quoted from Dardenne (1962)

We shall confine ourselves to a short

Table 53. Increased prevalence of diabetic retinopathy since the introduction of insulin therapy (after Dardenne 1962)

Author	Country	Year	Number of diabetics	Cases with diabetic retinopathy	
				Number	Percentage
Wagener & Wilder	U.S.A.	1921	300	44	14.7
Gray	Great Britain	1933	500	66	13.2
Wagener *et al.*	U.S.A.	1934	(1 952)	187	17.7
Waite & Beetham	U.S.A.	1935	2 002	372	18.0
Braun	Germany	1937	697	115	16.5
Hanum	Denmark	1939	966	195	20.2
Mc Kee	Canada	1941	2 350	476	20.2
Wagener	U.S.A.	1945	1 021	312	30.6
Barnes	U.S.A.	1950	220	80	36.0
Heinsius	Germany	1952	620	203	32.5
Appel *et al.*	Germany	1952	338	130	38.6
Aarseth	Norway	1953	288	121	42.0
Scott	Great Britain	1953	150	60	40.0
Porstmann & Wiese	Germany	1954	720	326	45.3
Dollfu	France	1954	1 303	681	52.4
Cowan *et al.*	U.S.A.	1955	500	216	43.2
Kornerup	Sweden	1955	1 000	468	46.8
Palomar	Spain	1956	416	175	42.0
Ashton	Great Britain	1957	203	137	67.0

review of the paper by Kornerup (1955) mentioned in the table. Kornerup studied the occurrence of Rd among 518 male and 482 female diabetics treated at certain Stockholm clinics during the years 1945–53. The patients are classified by age at onset (0–14, 15–40, 41–) and the material is divided into 5 year groups by the duration of DM.

In those cases where Kornerup did not find retinopathic changes by direct ophthalmoscopy in mydriasis, the patients were re-examined in monochromatic light with a wavelength of 5 720 Å. Of the 988 cases where the fundi could be examined with certainty, 462 or 46.8 per cent had some form of Rd. At the time this figure was considered high compared

Table 54. Percentage frequency of diabetic retinopathy by sex, age at onset and duration (after Kornerup 1955)

Duration	Males				Females			
	Age at onset			All	Age at onset			All
	0–14	15–40	41–		0–14	15–40	41–	
0	0	0	9	4	0	3	7	5
1–4	29	18	9	15	16	7	17	13
5–9	67	53	42	53	25	50	46	43
10–14	82	73	67	76	80	72	77	76
15–19	90	93	73	89	65	90	78	77
20–24	79	70	83	77	85	85	75	83
25–	86	85	25	75	75	100	–	83
All	74	49	31	51	54	42	36	42

with other investigations for instance that by Aarseth (1953) giving 42 per cent and that by Engleson (1954) giving 33 per cent Kornerup indicates the severity of Rd with the code numbers 0 for no changes 1 for haemorrhagic 2 for exudative and 3 for proliferative changes these codes have been used also in our clinical series He makes the important observation that fundus hypertonicus and Rd are two different and independent morbid states For later comparison Kornerup s Table V is reproduced (our Table 54)

Many other works deserve to be mentioned Often, however the published data are not sufficiently extensive to admit comparisons Some works treat only special forms of Rd this applies for instance to the paper by Root Mirsky and Ditzel (1959) where 847 cases of proliferative Rd are studied among 44 249 patients of the Joslin Clinic

In certain cases Rd causes blindness The number of diabetically blind persons in Sweden is not known In the U S A the number has been estimated at 29 000 making 8 4 per cent of all blind persons and about 1 per cent of all known diabetics Estimating the number of Swedish diabetics at 159 000 (cf p 114) we should thus expect about 1 600 diabetically blind persons in Sweden In 1963 the Swedish Association of the Blind had 737 persons registered as diabetically blind, it considers that this number represents about 50 per cent of all diabetically blind persons in Sweden If we draw the conclusion that the total number exceeds 1 500 the great importance of this sociomedical problem is evident

Diabetic retinopathy in the hospital series

From the case records the particulars regarding retinopathy were converted into the corresponding occasion card according to the code used by Kornerup (1955)

0 = no sign of Rd
1 = haemorrhagic Rd
2 = exudative Rd
3 = proliferative Rd

Departments of ophthalmology were created at Falun in 1922 at Östersund in 1940 and at Vänersborg in 1947, but at Växjo not until 1955 It was therefore not to be expected that the registrations for different years and hospitals should be consistent Our misgivings in this respect were later confirmed

The diagnoses covered by the codes 1 2 and 3 do not represent an irreversible progress in time or a strict gradation according to severity but for several reasons we have treated the data as if this were the case If any of the occasion cards forming the basis of a duration period of five years contained codes for positive findings of Rd the highest of these codes was carried to the duration card for this period If for a patient Rd had been diagnosed and coded in an earlier duration period the code was carried to his succeeding duration card not only if no ophthalmoscopy had been made during this latter period but also if absence of Rd had been reported by the code 0, or the Rd had been denoted by a lower code number

The motive for adopting this principle was mainly that for many mild cases some duration cards were based on one

Table 55 Registered state of retinopathy by sex age at onset duration epoch and

O D E	Hospital and state of retinopathy																					
	Vänersborg P				Växjö G				Falun W				Östersund Z				Total					
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0-3	1-3
Males																						
J a 1	43	–	–	–	28	–	–	–	19	–	–	–	27	–	–	–	117	–	–	–	117	–
2	4	–	–	–	5	–	–	–	4	–	–	–	3	–	–	–	16	–	–	–	16	–
b 1	30	1	–	–	21	–	–	–	20	1		–	17	–	–	–	88	2	–	–	90	2
2	11	1	–	–	9	2	–	–	8	–	–	–	5	–	–	–	33	3	–	–	36	3
c 1	[illegible]	2	–		10	2	–	1	13	4	–	–	14	–	–	–	55	8	–	1	64	9
2	13	7	–	–	11	2		–	9	3	–	1	9	2	–		42	14	–	1	57	15
d 1	11	4		–	6	1		–	7	1	–	–	7	–	–	–	31	6	–	–	37	6
2	6	13	1		4	3	3		4	10		1	4	1	–	–	18	27	4	1	50	32
e 1	2	1		–	2	–			1	1		–	2	–	–	–	7	2	–	–	9	2
2	4	10	1		1	2	1	–	4	9	–	2	3	1	1	–	12	22	3	2	39	27
f 1	3	1				–				1	–		1	–	–	–	4	2	–	–	6	2
2	1	6	2	1	3		2	1	2	4	–	–	4	1	–	–	10	11	4	2	27	17
A a 1	78				47			–	49	1	1		69	–	–	–	243	1	1	–	245	2
2	36	1			19			–	64	–	–	–	20	1	–	–	139	2	–	–	141	2
b 1	47				28			–	23	3	–	–	26	–	–	–	124	3	–	–	127	3
2	34	5			17	2			37	6	1	–	21	2	–	–	109	15	1	–	125	16
c 1	27	1			16	3		–	16	2	2	–	14	2	1	–	73	8	3	–	84	11
2	16	12	[illegible]	1	7	2	2		20	10	1		13	3	–	–	56	27	5	1	89	33
d 1	13				8	6	1		6	2	1		4	–	1	–	31	8	3	–	42	11
2	9	13	4		4	4	4	1	11	14		–	6	9	3	–	30	40	11	1	[illegible]	52
e 1	6	3			2				2			–	2	1	–	–	12	4	–	–	16	4
2	7	7	1		2	8	6		6	7	2	–	4	2	3	–	19	24	12	–	55	36
f 1	3					1			13	1		–	2	1		–	18	3	–	–	21	3
2	14	7	2	1	1	3	2	1	9	4	1	–	3	–		–	27	14	5	2	48	21
L a 1	95	2	1		79				66	3		–	169	2	2	–	409	7	3	–	419	10
2	123	10	3	–	84	1	1		136	7	2		146	10	2		489	28	8	–	525	36
b 1	33	1	1		22	1	1		27	2		–	58		1	–	140	4	3	–	147	7
2	77	15	2		53	1	1		73	9	2		78	10	1	–	281	35	6	–	322	41
c 1	19	1		1	11		1		13	4	1	–	26	2	–		69	7	2	1	79	10
2	21	17	3	2	16	1	3		25	8	7		21	8	1		[illegible]	34	14	2	133	40
d 1	3				6				5				12	–		–	26	–	–	–	26	–
2	16	9	5	2	7		1		11	9	3	1	6	4		1	40	22	9	4	75	35
e 1	2				2	1			1				4				9	1	–	–	10	1
2	7	3			4		1		5	2		–	4	1		–	20	6	1	–	27	7
f 1	2	1							1						–	–	3	1	–	–	4	1
2	3				2				2		–			1	–		7	1	–	–	8	1

State of Rd 0 no findings
1 haemorrhagic
2 exudative
3 proliferative

Age at onset (O) J 0-14
A 15-39
L 40-

or very few occasions often without connection with the diabetes To accept these registrations where no examination by an ophthalmologist had been made would probably introduce more errors in the data for calculating the incidence and prevalence of Rd than our simple procedure does

	Hospital and state of retinopathy																					
O D E	Vänersborg P				Växjö [illegible]				Falun W				Östersund Z				Total					
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0–3	1–3
Females																						
J a 1	37	–	–	–	27	–	–	–	30	–	–	–	27	–	–	–	121	–	–	–	121	–
2	2	–	–	–	1	–	–	–	1	–	–	–	–	–	–	–	4	–	–	–	4	–
b 1	27	2	–	–	21	1	–	–	15	–	–	–	20	–	–	–	83	3	–	–	86	3
[illegible]	7	1	–	–	7	–	–	–	9	2	–	–	3	–	–	–	26	3	–	–	29	3
c 1	12	3	1	–	12	2	1	–	9	3	–	–	9	1	–	–	42	9	2	–	53	11
2	6	10	–	–	11	1	2	–	9	10	–	1	8	3	–	–	34	24	2	1	61	27
d 1	5	1	1	–	6	–	–	–	7	1	1	1	1	1	2	–	19	3	4	1	27	8
2	2	10	2	–	4	1	3	–	3	4	1	2	6	2	1	–	15	17	7	2	41	26
e 1	1	1	–	–	1	1	–	–	3	3	1	–	–	–	–	–	5	5	1	–	11	6
2	3	3	2	–	2	4	–	–	1	5	3	1	2	–	–	–	8	12	5	1	26	18
f 1	–	1	–	–	1	–	–	–	1	1	–	–	–	–	–	–	2	2	–	–	4	2
2	4	5	1	–	1	–	2	–	2	4	1	1	1	–	–	–	8	9	4	1	22	14
A a 1	43	1	–	–	27	–	–	–	47	–	–	–	30	–	–	–	147	1	–	–	148	1
2	29	–	–	–	14	–	1	–	33	2	–	–	24	–	–	–	100	2	1	–	103	3
b 1	22	3	–	–	13	3	–	–	27	3	–	–	21	2	–	–	83	11	–	–	94	11
2	21	8	–	–	14	2	1	–	33	2	–	–	11	–	–	–	79	12	1	–	92	13
c 1	8	1	–	–	14	3	–	–	18	3	–	–	13	1	1	–	53	8	1	–	[illegible]	9
2	9	15	2	–	7	3	1	–	14	9	1	–	6	2	–	–	36	29	4	–	69	33
d 1	5	1	–	–	8	1	1	–	14	3	–	–	7	–	1	–	34	5	2	–	41	7
2	2	8	1	–	4	5	1	–	6	7	2	–	8	2	1	–	20	22	5	–	47	27
e 1	3	–	1	–	3	–	1	–	6	3	–	–	2	2	–	–	14	5	2	–	21	7
2	2	4	1	–	5	1	3	–	9	7	1	–	3	1	–	–	19	13	5	–	37	18
f 1	5	–	–	–	2	–	–	–	3	–	–	–	–	–	–	–	10	–	–	–	10	–
2	8	4	1	–	9	1	–	–	9	7	2	–	5	2	–	–	31	14	3	–	48	17
L a 1	123	5	–	–	165	3	2	–	112	3	1	–	237	7	4	1	637	18	7	1	663	26
2	188	19	1	–	111	1	4	–	179	15	2	–	210	21	9	–	688	56	16	–	760	72
b 1	36	6	–	–	64	1	1	–	39	6	–	–	80	5	2	–	219	18	3	–	240	21
2	127	17	4	1	97	9	3	–	124	30	2	–	101	22	5	–	449	78	14	1	542	93
c 1	17	–	–	–	34	4	2	–	16	8	1	–	36	5	1	–	103	17	4	–	124	21
2	30	26	1	2	35	5	3	–	41	26	4	–	25	14	4	–	131	71	12	2	216	85
d 1	4	1	–	–	5	1	–	–	7	2	1	–	9	3	1	–	25	7	2	–	34	[illegible]
2	6	12	3	–	21	3	6	–	15	18	3	–	11	9	1	–	53	42	13	–	108	55
e 1	1	–	–	–	–	–	–	–	1	–	–	–	2	–	–	–	4	–	–	–	4	–
2	3	3	1	1	4	1	2	–	5	4	1	–	1	2	–	1	13	10	4	2	29	16
f 1	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	2	–
2	3	–	1	–	1	–	–	–	–	1	–	–	–	1	1	–	4	2	2	–	8	4

Duration (D) a 0–4, b 5–9, c 10–14, d 15–19, e 20–24, f 25–

Epoch (E) 1 duration periods beginning 1949 or earlier; 2 duration periods beginning 1950 or later

As the patients in two consecutive duration groups are not identical the registrations of retinopathic state in the duration cards are not sufficient to admit conclusions concerning the course of Rd in a general diabetic population For this reason the individual changes of state from one duration period to the

Table 62 Intensity of retinopathy by duration both sexes

Duration	Intensity of retinopathy (with haemorrhagic = 1 exudative = 2 proliferative = 3)			
	All diabetics		Retinopathic diabetics	
	Our series	Kornerup	Our series	Kornerup
0– 4*	0 09	0 17	1 6	1 2
5– 9	0 17	0 67	1 3	1 4
10–14	0 47	1 40	1 5	1 8
15–19	0 72	1 62	1 7	1 9
20–24	0 77	1 57	1 8	2 0
25–	0 63	1 43	2 0	1 9

* Kornerup 1–4 years

been allotted the weights 1 2 and 3 respectively Thus we obtain the comparison shown in Table 62

From the discussion in the preceding section it follows that we should expect lower intensities among all diabetics If however the differences were due mainly to incomplete registration in our series of the easy cases of haemorrhagic Rd our material ought to show a higher intensity among the retinopathic patients This is clearly not the case and the most probable explanation is that even in the later epoch our registrations regarding the different types are not as complete as those of Kornerup

For the later epoch Table 63 shows the registrations at the four hospitals compared with the expected values calculated from the frequencies for all hospitals combined From the table it can be seen that hospital G, with its low retinopathy frequency, has comparatively more advanced cases than the other hospitals, and that the two hospitals P and W with the highest overall frequencies have comparatively fewer advanced cases These facts support the conjecture that even among the diagnosed cases of Rd the registrations of the type of Rd have not been as uniform as intended

Retinopathy and control

The question of the influence of treatment and control on the course of the late complications has not been definitely answered

To get an idea of the factors which might be of interest in connection with this important problem we made a special calculation where for the durations 10–14 and 15–19 years the fre

Table 63 Standard comparison between hospitals of states of retinopathy in the later epoch both sexes

Hospital	Diagnosed type of retinopathy							
	Haemorrhagic		Exudative		Proliferative		All types	
	Exp	Obs.	Exp	Obs.	Exp	Obs.	Exp	Obs.
P	226 2	281	56 7	47	7 4	11	290 3	339
G	139 0	68	34 7	59	4 4	3	178 1	130
W	230 0	255	57 6	45	7 1	7	294 7	307
Z	145 8	137	35 0	33	4 1	2	184 9	172
Total	741 0	741	184 0	184	23 0	23	948 0	948

Table 64 Frequency of retinopathy by sex age at onset, hospital and treatment durations 10–14 and 15–19

	Duration [illegible]						Duration 15–19					
	Males			Females			Males			Females		
	Total	With Rd		Total	With Rd		Total	With Rd		Total	With Rd	
		number	per cent		number	per cent		number	per cent		number	per cent
Total material	506	128	25	585	186	32	312	136	44	298	132	44
Hospital												
Vanersborg P	163	49	30	143	61	43	109	51	47	64	40	62
Vaxjo G	88	17	19	140	27	19	59	24	41	70	22	31
Falun W	139	43	31	173	66	38	[illegible]	42	49	[illegible]	46	47
Östersund Z	116	19	16	129	32	25	58	19	33	66	24	36
Age at onset												
0–14	121	24	20	114	38	33	87	38	44	68	34	50
15–39	173	44	25	131	42	32	124	[illegible]	51	88	34	39
40–	212	60	28	340	106	31	101	35	35	142	64	45
Weight at onset												
normal	494	124	25	552	174	32	310	135	44	294	130	44
overweight*	12	4	33	33	12	36	2	1	50	4	2	50
Diet regulated												
strictly	64	20	31	73	24	33	26	11	42	23	16	70
not strictly	442	108	24	512	162	32	286	125	44	275	116	42
Daily dose of insulin												
0–39 I U	166	46	28	249	71	29	81	29	36	115	55	48
40– I U	340	82	24	336	115	34	231	107	46	183	77	42

* Compared with the normal weight according to Broca (weight in kg equal to length in cm minus 100) the following excess weight has been considered as overweight

Age at onset	*Duration 10–14*	*Duration 15–19*
0–34	5 kg or more	10 kg or more
35–	10 kg or more	15 kg or more

quencies of Rd were studied in groups by hospital and age at onset and with simultaneous dichotomizations regarding weight at onset diet and daily insulin dose The limitation to the two dura tions 10–14 and 15–19 years was warranted by the comparatively few cases of Rd in the lower durations and the low number of patients in the higher dura tions The resulting table did not show any tendencies differing from normal random fluctuations As the table is rather extensive an extract showing only some marginal frequencies is given in Table 64 From the table it can be clearly seen that the differences according to treatment are insignificant in comparison with the large differences between hospitals which differences have been discussed earlier in this chapter

Either from the complete table or from the extract (Table 64) it is possible to prove or disprove connections between treatment and the development of retinopathy Nevertheless *we think that the* data support our belief that even if such

connections exist no treatment hitherto used in general hospitals will decisively improve or retard the retinopathic changes among the diabetics

Diabetic nephropathy (Pd)

The diagnosis diabetic nephropathy (Pd) is partly clinical partly patho-anatomical[1]

The patho-anatomical designation is limited to renal lesions, caused by those capillary changes characteristic of diabetes which are now termed diabetic microangiopathy (cf Rifkin & Leiter 1962) It should be remembered that this renal disorder Kimmelstiel Wilson s syndrome can hardly be diagnosed clinically

Through studies of biopsies it is known that signs of diabetic nephropathy may appear long before the diagnosed onset of DM and without any albuminuria being present

If as in this study the occurrence of persistent albuminuria is considered as the clinical manifestation of Pd it is obvious that the complication will be diagnosed at a comparatively advanced stage

Studies on the prevalence of diabetic nephropathy have been made among others by Mårtensson (1950) and Johnsson (1959 1960) Alwall Ekelund and Oras (1950) have investigated certain problems connected with intercapillary glomerulosclerosis (Kimmelstiel Wilson s syndrome) Among Swedish investigations of the renal function in diabetic nephropathy mention may be made in particular of those by Hogeman (1948) and by Bucht Ek and Werkö (1956)

Hogeman (1948) made a thorough study of the renal function in 12 patients with uncomplicated DM and 12 patients who had diabetic nephropathy with proteinuria and positive urinary sediment as well as elevated blood pressure and retinal changes Renal function in uncomplicated DM was found to be normal For the patients with nephropathy there was a change in renal function towards pathologically decreased values the same results were found in non-diabetic patients with malignant hypertension Despite this similarity the patients with nephropathy showed a better prognosis than those with malignant hypertension The insulin clearance the diodrast clearance and the filtration fraction (the relation between the insulin and diodrast clearances) were low in diabetic nephropathy whereas on the contrary a high filtration fraction is a common phenomenon in nephrosclerosis and hypertension The author is of opinion that the mechanism causing the decrease in renal function is entirely different and that the malignant phase of diabetic nephropathy consists of intercapillary glomerulosclerosis

Diabetic nephropathy in the hospital series

In the occasion cards the following states of Pd were coded

0 = no persistent albuminuria,
1 = persistent albuminuria without signs of renal insufficiency
2 = renal insufficiency

The term *persistent albuminuria* signifies here that albuminuria had been observed *on three consecutive occasions* In the preceding section it was pointed out

[1] Cf e.g Berkman (1962) and Thomsen (1965)

that the adoption of the occurrence of persistent albuminuria as a criterion for the diagnosis diabetic nephropathy will imply a comparatively late diagnosis Our definition of the concept persistent albuminuria will further delay the registration of Pd for patients who seldom visit the hospitals For patients who have been admitted only once or twice even a pronounced albuminuria will escape classification as Pd We must thus expect to register a lower prevalence and a later incidence of Pd than are usually found in investigations made by specialists with large resources

From the occasion cards belonging to one duration period, the highest code occurring has been transferred to the corresponding duration card

Prevalence of nephropathy among DM patients

The states of Pd as registered in the duration cards are shown in the basic Table 65 From these data the prevalence of Pd during the total investigation period has been calculated in Table 66

Table 66 of the nephropathy prevalence shows main features resembling those of the retinopathy prevalence in Table 56 The frequency of renal disorders has a maximum after 15–24 years duration and shows a slight decrease in the highest duration group Of course this does not imply that a diabetic with Pd may expect a recovery from his renal disorder after 25 years duration The decrease is mainly due to the selection caused by the death of patients with renal insufficiency or a generally malignant course of DM This selection is, however obscured by a lack of homogeneity in the registrations for different hospitals and during the period studied As in the case of retinopathy, we shall therefore study the incidence of Pd and the inhomogeneities just mentioned

Incidence of nephropathy

The changes in the state of Pd between consecutive duration periods were registered according to the code defined earlier in respect of Rd For Pd the main results are shown in Table 67 A striking feature in the table is the recoveries registered in 144 duration cards (73 males and 71 females) That so many diabetics with permanent albuminuria in one duration period should be free from this symptom during the following period seems to be unlikely The recoveries are probably due to incomplete registration This remark seems valid also for the two instances of female diabetics registered as free from earlier renal insufficiency

From Table 67 can be calculated the incidence of Pd, as shown by the following example for males with age at onset 0–14 years (type J) during duration 5–9 years (duration b) Out of the 111 diabetics observed during the previous duration period also 92 have shown no Pd whilst 7 earlier unaffected patients have had Pd diagnosed in the current duration period The incidence rate for this group of patients is thus 7 divided into 99 or 7 1 per cent If the incidence rate were interpreted as the risk of a diabetic having Pd

Table 65 · Registered state of nephropathy by sex age at onset duration epoch and

O	D	E	Vänersborg P 0	Vänersborg P 1	Vänersborg P 2	Växjö G 0	Växjö G 1	Växjö G 2	Falun W 0	Falun W 1	Falun W 2	Östersund Z 0	Östersund Z 1	Östersund Z 2	Total 0	Total 1	Total 2	Total 0-2	Total 1-2
			Hospital and state of nephropathy																
Males																			
J	a	1	33	10	–	28	–	–	16	3	–	27	–	–	104	13	–	117	13
		2	4	–	–	5	–	–	4	–	–	3	–	–	16	–	–	16	–
	b	1	24	7	–	20	1	–	15	6	–	17	–	–	76	14	–	90	14
		2	9	3	–	10	1	–	8	–	–	5	–	–	32	4	–	36	4
	c	1	12	8	–	9	3	1	13	4	–	12	2	–	46	17	1	64	18
		2	11	9	–	8	5	–	12	1	–	11	–	–	42	15	–	57	15
	d	1	10	5	–	6	1	–	6	2	–	4	2	1	26	10	1	37	11
		2	10	9	1	4	6	–	9	4	2	5	–	–	28	19	3	50	22
	e	1	2	1	–	1	1	–	–	1	1	2	–	–	5	3	1	9	4
		2	7	6	2	2	2	–	9	4	2	4	1	–	22	13	4	39	17
	f	1	4	–	–	–	–	–	–	1	–	1	–	–	5	1	–	6	1
		2	7	3	–	5	1	–	4	1	1	4	1	–	20	6	1	27	7
A	a	1	64	13	1	46	1	–	49	2	–	68	1	–	227	17	1	245	18
		2	34	3	–	19	–	–	56	8	–	20	1	–	129	12	–	141	12
	b	1	42	5	–	27	1	–	23	3	–	25	1	–	117	10	–	127	10
		2	36	3	–	18	1	–	41	3	–	23	–	–	118	7	–	125	7
	c	1	23	5	–	16	3	–	18	1	1	17	–	–	74	9	1	84	10
		2	27	2	2	8	1	2	26	3	2	16	–	–	77	6	6	89	12
	d	1	11	2	–	11	3	1	8	1	–	4	1	–	34	7	1	42	8
		2	18	8	–	7	2	4	16	7	2	14	1	3	55	18	9	82	27
	e	1	7	2	–	2	–	–	2	–	–	3	–	–	14	2	–	16	2
		2	8	6	1	10	4	2	13	1	1	8	1	–	39	12	4	55	16
	f	1	2	1	–	1	–	–	13	1	–	3	–	–	19	2	–	21	2
		2	18	6	–	4	2	1	11	3	–	3	–	–	36	11	1	48	12
L	a	1	84	14	–	79	–	–	61	6	2	171	2	–	395	22	2	419	24
		2	102	34	–	82	3	1	128	14	3	153	2	3	465	53	7	525	60
	b	1	29	6	–	22	2	–	28	1	–	58	1	–	137	10	–	147	10
		2	71	21	2	49	3	3	70	13	1	86	3	–	276	40	6	322	46
	c	1	16	5	–	11	1	–	15	3	–	27	1	–	69	10	–	79	10
		2	32	10	1	18	2	–	33	5	2	27	2	1	110	19	4	133	23
	d	1	1	2	–	5	1	–	3	1	1	11	1	–	20	5	1	26	6
		2	20	9	3	8	–	–	18	6	–	10	1	–	56	16	3	75	19
	e	1	1	1	–	3	–	–	1	–	–	4	–	–	9	1	–	10	1
		2	8	2	–	4	1	–	5	2	–	5	–	–	22	5	–	27	5
	f	1	3		–	–	–	–	–	1	–	–	–	–	3	1	–	4	1
		2	2	1	–	2	–	–	2	–	–	1	–	–	7	1	–	8	1

State of Pd: 0 no persistent albuminuria; 1 persistent albuminuria; 2 renal insufficiency

Age at onset (O): J 0-14; A 15-39; L 40

for the first time during the duration period the denominator should properly be corrected for the reduction of observation time caused by those of the 99 who have left the investigation before the end of the duration period Disregarding this correction we confine ourselves to stating that the incidence rates calculated are on

[illegible]	D	E	Hospital and state of nephropathy																
			Vänersborg P			Växjö G			Falun W			Östersund Z			Total				
			0	1	2	0	1	2	0	1	2	0	1	[illegible]	[illegible]	1	2	0–2	1–2
Females																			
J	a	1	25	12	–	26	1	–	26	4	–	27	–	–	104	17	–	121	17
		2	1	1	–	1	–	–	1	–	–	–	–	–	3	1	–	4	1
	b	1	18	11	–	21	1	–	11	4	–	20	–	–	70	16	–	[illegible]	16
		2	5	3	–	7	–	–	10	–	–	3	–	–	25	4	–	29	4
	[illegible]	1	8	8	–	11	4	–	7	5	–	10	–	–	36	17	–	53	17
		2	9	7	–	13	1	–	13	4	3	10	1	–	45	13	3	61	16
	d	1	3	3	1	3	3	–	4	4	2	2	1	1	12	11	4	27	15
		2	7	6	1	6	2	–	3	3	4	8	–	1	24	11	6	41	17
	e	1	–	2	–	2	–	–	1	5	1	–	–	–	3	7	1	11	8
		2	5	1	2	2	3	1	6	3	1	2	–	–	15	7	4	26	11
	f	1	–	1	–	–	1	–	1	1	–	–	–	–	1	3	–	4	3
		2	9	1	–	1	1	1	4	3	1	1	–	–	15	5	2	22	7
A	a	1	32	12	–	27	–	–	37	10	–	29	1	–	125	23	–	148	23
		2	19	10	–	15	–	–	34	1	–	24	–	–	92	11	–	103	11
	b	1	20	5	–	15	1	–	24	5	1	23	–	–	82	11	1	94	12
		2	23	6	–	17	–	–	35	–	–	11	–	–	86	6	–	92	6
	c	1	9	–	–	16	1	–	13	7	1	13	1	1	51	9	2	62	11
		2	17	7	2	8	2	1	19	4	1	8	–	–	52	13	4	69	17
	d	1	5	1	–	7	3	–	10	6	1	7	1	–	29	11	1	41	12
		2	6	3	2	6	3	1	12	2	1	9	1	1	33	9	5	47	14
	e	1	2	1	1	2	2	–	6	2	1	3	1	–	13	6	2	[illegible]	8
		2	6	1	–	6	3	–	11	5	1	3	1	–	26	10	1	37	11
	f	1	5	–	–	2	–	–	2	1	–	–	–	–	9	1	–	10	1
		2	7	3	3	8	2	–	14	3	1	6	–	1	35	8	5	48	13
L	a	1	120	8	–	157	12	1	106	9	1	244	5	–	627	34	2	663	36
		2	158	[illegible]	2	113	2	1	165	28	3	233	6	1	669	84	7	760	91
	b	1	36	5	1	[illegible]	1	1	42	3	–	85	2	–	227	11	2	240	13
		2	105	39	5	100	5	4	138	[illegible]	2	125	3	–	468	[illegible]	11	542	74
	c	1	15	2	–	36	3	1	[illegible]	6	1	39	3	–	108	14	2	124	16
		2	[illegible]	19	2	38	4	1	60	9	2	41	2	–	177	34	5	216	39
	d	1	5	–	–	6	–	–	7	1	2	12	1	–	30	2	2	34	4
		2	11	10	–	22	4	4	25	9	2	20	–	1	78	23	7	108	30
	e	1	1	–	–	–	–	–	1	–	–	2	–	–	4	–	–	4	–
		2	4	3	1	6	1	–	6	4	–	3	1	–	19	9	1	29	10
	f	1	2	–	–	–	–	–	–	–	–	–	–	–	2	–	–	2	–
		2	4	–	–	–	1	–	–	1	–	2	–	–	6	2	–	8	2

Duration (D): a 0–4, b 5–9, c 10–14, d 15–19, e 20–24, f 25–

Epoch (E): 1 duration periods beginning 1949 or earlier; 2 duration periods beginning 1950 or later

the low side compared with the risks discussed

From these incidence rates the prevalence of Pd has been calculated under the unrealistic hypothesis that the incidence as well as the prevalence are the same for patients observed during two consecutive duration periods as for pa-

Table 66 Frequency of nephropathy by sex, age at onset and duration

Duration	Percentage frequency of nephropathy (states 1 and 2)							
	Males				Females			
	Age at onset			All	Age at onset			All
	0–14	15–39	40–		0–14	15–39	40–	
0– 4	10	8	9	9	14	14	9	10
5– 9	14	7	12	11	17	10	11	13
10–14	27	13	16	17	29	21	16	20
15–19	38	28	25	30	47	30	23	31
20–24	44	25	16	29	51	33	30	38
25–	24	20	17	21	38	24	20	28
All	23	13	12	14	27	18	12	15

tients entering or leaving the investigation. The calculation has been performed as described earlier in respect of Rd. The results are given in Table 68 and are compared with the prevalences observed according to Table 66.

The difference between the prevalence as calculated from the incidence rates and the observed prevalence implies that the frequency of Pd among patients leaving the investigation is higher than that of those remaining. In the similar situation in the case of retinopathy it was shown that this effect was not due to the mortality of the worst cases but could be explained by the progress in diagnostics and more complete registration. As will appear in the following section, the increase in prevalence of diagnosed Pd is of a much smaller magnitude than in respect of retinopathy. Unlike retinopathy, Pd is an important cause of death for diabetics. It is therefore probable that the difference between calculated and observed prevalence of Pd is due largely to the death of diabetics with pronounced nephropathy.

Table 68 gives the calculated incidence rates of nephropathy. Owing to the special purpose of this table, even the registrations of Pd at duration 0–4 years were used under the oversimplified hypothesis that persistent albuminuria does not occur before the onset of DM. The distributions of the new cases show some interesting characteristics. There is a high incidence rate of Pd at duration 0–4 years, followed by a fairly even distribution with a maximum at the durations 15–19 or 20–24 years. This picture might correspond to different diagnostic situations. In cases where albuminuria has been an indication of diabetes, it is evident that Pd will in general appear early in the registrations. In most cases the original admission and diagnosis have been connected with the occurrence of acidosis and glycosuria, and signs of albuminuria have gradually appeared.

These general characteristics of the incidence rates for Pd may to a certain extent reflect an uncertainty regarding the exact year of onset, with resulting vagueness in the definition of duration. The comparatively high percentage of well-defined retinopathy in the lowest duration group gives support to the

Table 67 Changes in the registered state of nephropathy by sex age at onset and duration

Sex and age at onset	Duration	0 No Pd	1 Recovery	2 Improvement	3 Unchanged	4 New Pd	5 Aggravation	– New patients	Total
		Type of change in state of nephropathy							
Males									
0–14	0– 4							133	133
	5– 9	[illegible]	4	–	8	7	–	15	126
	10–14	71	6	–	11	18	–	15	121
	15–19	43	5	–	16	13	2	8	87
	20–24	24	–	–	8	[illegible]	4	6	48
	25–	[illegible]	1	–	7	–	–	6	33
15–39	0– 4							386	386
	5– 9	204	15	–	4	13	–	16	252
	10–14	130	7	–	4	15	2	15	173
	15–19	78	4	–	8	19	2	13	124
	20–24	42	4	–	7	9	2	7	71
	25–	39	5	–	5	7	–	13	69
40–	0– 4							944	944
	5– 9	354	10	–	20	25	2	58	469
	10–14	151	6	–	8	19	2	26	212
	15–19	66	1	–	12	8	2	12	101
	20–24	24	1	–	5	1	–	6	37
	25–	6	4	–	–	1	–	1	12
Females									
0–14	0– 4							125	125
	5– 9	78	6	–	9	9	–	13	115
	10–14	66	5	–	13	15	1	14	114
	15–19	28	5	–	16	10	4	5	68
	20–24	13	3	–	10	2	2	7	37
	25–	10	3	–	7	–	1	5	26
15–39	0– 4							251	251
	5– 9	130	13	–	9	5	–	29	186
	10–14	90	4	–	8	16	1	[illegible]	131
	15–19	53	3	1	8	10	4	9	88
	20–24	33	2	–	11	6	1	5	58
	25–	30	3	1	5	3	3	13	58
40–	0– 4							1 423	1 423
	5– 9	585	17	–	20	53	3	104	782
	10–14	248	6	–	11	32	2	41	340
	15–19	91	1	–	10	19	2	[illegible]	142
	20–24	20	–	–	4	5	1	3	33
	25–	8	–	–	–	1	–	1	10

suspicion that especially in the case of late DM the year of first admission has sometimes been substituted for the unknown or uncertain year of onset

The codes of change used in Table 67 are not sufficiently specified to admit an evaluation of the first appearance of renal insufficiency as this appearance may be coded as either new or aggravated Pd The cases are too few to admit a reliable calculation of incidence rates but a special list was made from which Table

Table 68 *Calculated incidence and prevalence of nephropathy by sex, age at onset and duration*

Sex and age at onset	Duration	Patients with no Pd in earlier periods: Total number t	With Pd diagnosed in period r	Incidence of Pd $I_d = \frac{r}{t}$	Prevalence of Pd per cent: Calculated P_d	Observed (Table 66)
Males						
0-14	0- 4	133	13	0.0977	10	10
	5- 9	99	7	0.0707	15	14
	10-14	99	18	0.1818	31	27
	15-19	56	13	0.2321	47	38
	20-24	30	6	0.2000	57	44
	25-	18	–	–	57	24
15-39	0- 4	386	30	0.0777	8	8
	5- 9	218	13	0.0596	13	7
	10-14	145	15	0.1034	22	13
	15-19	97	19	0.1959	37	28
	20-24	51	9	0.1765	48	25
	25-	46	7	0.1522	56	20
40-	0- 4	944	84	0.0890	9	9
	5- 9	379	25	0.0660	15	12
	10-14	170	19	0.1118	24	16
	15-19	73	8	0.1096	33	25
	20-24	25	1	0.0400	35	16
	25-	7	1	0.1429	45	17
Females						
0-14	0- 4	125	18	0.1440	14	14
	5- 9	87	9	0.1034	23	17
	10-14	81	15	0.1852	37	29
	15-19	38	10	0.2632	54	47
	20-24	15	2	0.1333	60	51
	25-	10	–	–	60	38
15-39	0- 4	251	34	0.1350	14	14
	5- 9	135	5	0.0365	17	10
	10-14	106	16	0.1509	29	21
	15-19	63	10	0.1587	40	30
	20-24	39	6	0.1538	50	33
	25-	33	3	0.0909	54	24
40-	0- 4	1 423	127	0.0892	9	9
	5- 9	638	53	0.0831	16	11
	10-14	280	32	0.1143	26	16
	15-19	110	19	0.1727	39	23
	20-24	25	5	0.2000	51	30
	25-	9	1	0.1111	57	20

69 is reproduced to be compared with the column 'With Pd diagnosed in period' in Table 68. Thus new patients in durations above 0-4 years are not included as we do not know when the recorded insufficiency appeared. Comparing the figures with the change codes 4 and 5 in Table 67 it is remarkable that in many cases insufficiency is recorded without preceding registration of persistent albuminuria. The existence in the lowest duration group of 18 cases of

Table 69 First appearance of renal insufficiency by sex, age at onset and duration

Duration	Males (registrations)				Females (registrations)			
	Age at onset			All	Age at onset			All
	0–14	15–39	40–		0–14	15–39	40–	
0– 4	–	–	9	9	–	–	9	9
5– 9	–	–	4	4	–	–	10	10
10–14	–	7	4	11	2	5	2	9
15–19	3	5	3	11	7	5	7	19
20–24	4	3	–	7	2	1	1	4
25–	–	1	–	1	1	1	–	2
Total	7	16	20	43	12	12	29	53

renal insufficiency supports the above-mentioned suspicions about the exactitude of the year of onset.

Nephropathy frequency with regard to hospitals and epochs

From the exact frequencies behind the rounded off percentages in Table 66 we have calculated the number of nephropathy diagnoses which would have been expected if the registered prevalence had been the same at all hospitals and in the two epochs studied. For each sex the expected numbers for the four hospitals and the two epochs have been compared with the observed numbers. The quotients observed/expected or the (standardized indices) are shown in Table 70.

The difference in registered prevalence between the two epochs is illustrated by the increase of the standardized index from 83 to 112 per cent for males and from 86 to 110 per cent for females. Roughly speaking the registered prevalence has gone up by about 30 per cent.

In view of our experiences regarding the completeness of old case record registrations we are not prepared to conclude that the prevalence of Pd among diabetics has changed. As a certain number of patients observed in the earlier epoch are survivors from the pre-insulin era they may represent a selected group of comparatively mild cases of DM. The increase in Pd could thus represent the adaptation to a higher level corresponding to the reduction in diabetes mortality. The time variation is however insignificant compared with the almost startling differences between the hospitals, the standardized nephropathy indices being at Östersund (Z) 28, at Växjo (G) 71, at Falun (W) 114, and at Vänersborg (P) 166 per cent. Thus the prevalence of Pd at Vänersborg is found to be about six times the prevalence at Östersund.

The occurrence of different nephropathy states

In order to get a more complete picture the standard comparison has been performed separately for each of the two states persistent albuminuria and renal insufficiency. As the figures for males and females show identical patterns we confine ourselves to giving the main results for both sexes taken together as

Table 70 Standard comparison of nephropathy frequency by sex hospital and epoch

Sex and hospital	Number of patients (registrations) showing nephropathy								
	Epoch						Total		
	E1 (1949)			E2 (1950-)					
	Exp	Obs.	O E %	Exp	Obs.	O E %	Exp	Obs.	O E %
Males									
P	59.3	88	148	89 7	147	164	149 0	235	158
G	39 7	20	50	46 9	47	100	86 6	67	77
W	42 0	42	100	81 4	91	112	123 4	133	108
Z	55 1	13	24	53 9	20	37	109 0	33	30
Total	196 1	163	83	271 9	305	112	468 0	468	*100*
Females									
P	55 1	74	134	95 1	188	198	150 2	262	174
[illegible]	59 3	36	61	66 8	48	72	126 1	84	67
W	64 0	[illegible]	131	106 8	118	110	170 8	202	118
Z	68 1	18	26	70 8	20	28	138 9	38	27
Total	246 5	212	86	339 5	374	110	586 0	586	*100*
Both sexes									
[illegible]	114 4	162	142	184 8	335	182	299.2	497	166
G	99 0	56	57	113 7	95	84	212.7	151	71
W	106 0	126	119	188 2	209	111	294.2	335	114
Z	123.2	31	25	124 7	40	30	247 9	71	[illegible]
Total	442.6	375	85	611 4	679	111	1 054 0	1 054	100

shown in Table 71 The high prevalence figures at hospital P are dominated by cases of albuminuria whilst the subnormal prevalence of nephropathy at hospital G conceals a high standardized frequency of renal insufficiency This inconsistency is very difficult to explain by reference to different selection or

Table 71 Standard comparison of states of nephropathy by hospital and by epoch

Hospital Epoch	Number of patients (registrations) showing nephropathy								
	Persistent albuminuria without renal insufficiency			Renal insufficiency			Total nephropathy		
	Exp	Obs.	O E %	Exp	Obs.	O E %	Exp	Obs.	O E %
Hospital									
P	260 8	461	177	38 4	36	[illegible]	299.2	497	166
G	184 8	119	64	27 9	32	114	212.7	151	71
W	253 5	281	111	40 7	54	133	294.2	335	114
Z	217 9	56	26	30 0	15	50	247 9	71	[illegible]
Epoch									
E1 (1949)	393 9	347	88	45 7	28	58	442.6	375	85
E2 (1950-)	523 1	570	109	88 3	109	123	611.4	679	111
Total	917 0	917	*100*	137 0	137	*100*	1 054 0	1 054	100

different treatment of the patients In contrast with these hospitals the hospitals W and Z show for both states of Pd and for both epochs frequencies which are consistently high (W) or low (Z) Whether this difference is caused by different selection of patients by different treatment or by varying accuracy in diagnosis and case record registration it is highly remarkable that differences of the magnitude shown in Table 71 can occur between Swedish county hospitals headed by DM specialists during a period with no discontinuities or marked divergences in opinion regarding the treatment of DM

Diabetic neuropathy (Ud)

Compared with retinopathy and nephropathy, the diabetic neuropathy (Ud) is difficult to diagnose The symptoms are generally not sufficiently clear cut to admit a positive statement that a diabetic does or does not suffer from Ud There is no general agreement about the genesis and the patho anatomical picture of this complication or about its treatment

Schrader and Weinges (1961) surveyed the peripheral neurological disorders among diabetics some of which were reported as early as in 1864 by de Calvi The reported prevalence of Ud among diabetics varied between 10 and 100 per cent The variation is probably due to varying definitions of the disease varying investigation techniques and differently selected patients The authors estimated the prevalence of neuropathy among diabetics at about 50-60 per cent Their paper contains a fairly complete bibliography

Smith (1961) reported frequencies of Ud varying between 5 and 24 per cent among 1 200 diabetic hospital patients

In his study of neuropathy and diabetic control Ellenberg (1960) sums up as follows

1 Neuropathy may occur during good control

2 There may be simultaneous onset of neuropathy and the symptoms of uncontrolled glycosuria

3 The neuropathy is unrelated to the duration or severity of the diabetes

4 Neuropathy may be the initial clinical manifestation of diabetes unattended by symptoms of hyperglycemia and glycosuria

5 The paradoxic precipitation of neuropathy following institution of good control by diet insulin and tolbutamide has been observed

6 Neuropathy may follow stress situations in these instances a relatively constant latent period exists

In a later paper Ellenberg (1961) has maintained that absent deep reflexes is a diagnostic clue in unsuspected diabetes (cf also Ellenberg 1964, 1966) It seems probable that Ellenberg s assertions regarding the early diagnosis of DM and the influence of the treatment on the development of neuropathy are correct However even today the views of experts on DM are not unanimous [1]

Somlo, Csapo and Szucs (1962) found 55 per cent Ud among 500 diabetics although the material gives no information about age or duration Dolman (1963) estimated the Ud frequency re

[1] Fagerberg, Petersén Steg and Wilhelmsen (1963) emphasize that motor disturbances are common in diabetic neuropathy and that their frequency rises with the duration of DM as well as with the degree of diabetic angiopathy

Table 75 Standard comparison between hospitals of diagnosis of neuropathy both sexes

Hospital	Type of diagnosis							
	Subjective		Objective		Subj. and obj		All types	
	Exp	Obs.	Exp	Obs.	Exp	Obs.	Exp	Obs.
P	191.2	63	41 9	21	19 3	22	252 4	106
[redacted]	141 9	23	31 3	17	14 9	9	188 1	49
W	190 9	361	42 3	54	19 7	5	252 9	420
Z	193 0	270	42.5	66	20 1	38	255 6	374
Total	717 0	717	158 0	158	74 0	74	949 0	949

(2) or subjective and objective symptoms simultaneously (3) For each hospital the relative occurrence of the different codes showed the same picture for males and females and for the two epochs The differences between the hospitals are therefore illustrated in Table 75 based on the combined data

The varying occurrence of the different codes of diagnosis is not in itself surprising The need for case record data on subjective symptoms when objective symptoms have been found and *vice versa* may be differently judged even by careful doctors Thus the Ud diagnoses have been based upon subjective symptoms to a greater extent at hospital W than at hospital Z but the total frequencies are of the same order of magnitude In hospitals P and G on the other hand very few diagnoses are based on subjective symptoms alone indicating a more restrictive attitude towards the diagnosing of mild or undecided cases as instances of Ud

Assuming that the data from hospitals W and Z in the later epoch are based on comparatively complete records Table 76 computed from Table 72, may be considered as a maximum estimate of the neuropathy prevalence about 1955 at hospitals where even mild subjective

Table 76 . Frequency of neuropathy observed at hospitals W and Z in the later epoch by sex age at onset and duration

Duration	Percentage frequency of neuropathy							
	Males				Females			
	Age at onset			All	Age at onset			All
	0 14	15 39	40		0 14	15 39	40-	
0- 4		7	[redacted]	23		10	23	22
5- 9		7	34	25		13	27	25
10 14	4	19	36	25	19	41	35	33
15 19	30	33	37	34	42	27	[redacted]	34
20-24	40	29		[redacted]		24		34
25-				29		32		32
All	19	17	31	26	25	22	27	26

symptoms are considered as expressions of neuropathy Although the data in some groups are too limited to permit the calculation of percentages a comparison with Table 73 shows that these estimates of the frequency of neuropathy are practically twice as large as the frequencies registered for the total series With the concept adopted at hospitals W and Z the overall frequency of neuropathy among diabetic patients would have been about 26 per cent Nevertheless this frequency is far below some figures given in the literature

Osteopathy

Diabetic osteopathy may be regarded as a syndrome within the clinical picture of diabetic neuropathy, with trophic ulcers and various changes of the foot skeleton (cf p 31) Osteitis osteomyelitis osteoarthritis, Charcot joints and in some cases osteoporosis may be present

Diabetic osteopathy and diabetic gangrene may present difficult diagnostic problems The following table gives a schematic picture in respect of the clinical characteristics

Symptoms	Diabetic osteopathy	Diabetic gangrene
Pulse (arteria dorsalis ped)	Generally normal	Often lacking
Oscillometry	Normal	Low amplitudes
Skin temperature	Normal	Cold feet and toes
X ray of foot skeleton	Osteoporosis	Normal
Claudicatio intermittens	Rarely found	Frequently observed
Sensibility	Generally low or lacking	Vibration sensitivity often reduced
Pains	None	Severe

At the annual meeting of the American Diabetes Association in 1966, one of us (Gronberg 1966a) described four female diabetics with osteopathy (cf also Grönberg 1966)

Clinical data	Patient (female)			
	(1)	(2)	(3)	(4)
Year of birth	1931	1929	1900	1918
Age at onset of DM	[illegible]	22	32	13
Control	Poor	Fair	Good	Good
Age 1966 (age at death)	†29	37	66	48
Working capacity		Good	Good	Good
Duration of DM at onset of				
retinopathy	14	7	14	20
neuropathy	16	10	17	22
nephropathy	[illegible]	–	–	–
trophic ulcers	24	12	16	31
bone lesions	16	12	24	33
Treatment (see below)		+	+	–

Arteriography oscillometry and plethysmography were not significantly abnormal in these patients

Patients (2) and (3) were treated with intra arterial infusion of D-ampicillin for ten days (4 grammes per day) heparin and ergotamine were added to the infusions The results were surprisingly good The chronic trophic ulcers of the two patients who had had lesions for several years showed complete healing without recurrence for several months

The syndrome of osteopathy is estimated to occur in about 5 per cent of all diabetics There is no correlation with occlusive vascular disease (cf p 31) Infections and traumata are the most prominent factors affecting the course of diabetic osteopathy

The problem of the cause of trophic ulcers has not yet been solved Loss of

pain sensitivity in the feet may be of importance in their development. Possibly, the skeletal lesions may parallel the ulceration (cf. Ellenberg 1964) and they might not, as is usually assumed, be sequelae to infections of the ulcers.

Very likely the reduced sensitivity to pain has the result that the patients with diabetic osteopathy often are far less incapacitated than would be expected in view of the severe character of the trophic ulcers and skeletal changes.

Vascular lesions

Under this heading are included pathological states, other than retinopathy (Rd) and nephropathy (Pd), which are regarded as caused by vascular changes. This category would include cardiovascular diseases such as coronary infarction, angina pectoris, cardiac insufficiency (not connected with organic heart lesion due to rheumatoid arthritis) and the like, and peripheral disturbances such as gangrene and angiospasm. Of course the group is rather heterogeneous but on the other hand it is obvious that a hospital series of the present type must be biased in so far as the first admission to the hospital after the onset of DM is to a fairly large extent connected not with the occurrence of DM but with the symptoms arising from the vascular lesion.

Since a proper evaluation of this bias is not possible, it is not justifiable to perform a detailed analysis of different subgroups.

The frequency of registered occurrence of vascular disease by sex and age is shown in Table 77.

Table 77. Frequency of vascular lesions by sex and age

Average age	Number of patients		With vascular lesions per cent	
	M	F	M	F
4	16	15	–	–
9	60	70	–	–
14	135	118	0.7	–
19	210	174	0.5	–
24	213	173	0.5	2.3
29	211	147	1.4	2.0
34	224	148	1.8	4.1
39	217	148	1.4	3.4
44	227	154	2.6	3.2
49	243	237	4.1	8.0
54	282	334	7.1	9.6
59	297	476	12.5	14.7
64	305	521	12.8	17.9
69	264	534	20.5	20.8
74	205	384	27.3	24.2
79	131	198	26.0	26.3
84–	44	62	15.9	33.9
Total	3 284	3 893	8.4	13.2

On the basis of the frequencies by sex and age, a standard calculation with division of the series by age at onset and duration was performed. As can be seen from Table 78, only slight deviations from pure age dependence were found.

In the literature it is often maintained that, although the vascular lesions are not specific for DM, they have a tendency to appear earlier in diabetics than in non-diabetics. Naturally it is very difficult to arrive at definite evidence for the correctness of such a statement—even in prospective studies of a population series. The only conclusion that can be drawn on the basis of our data is that they do not give rise to a supposition that vascular lesions found in diabetics should generally be regarded as sequelae of DM.[1]

[1] Cf. also the discussion on p. 163.

Table 78. Standard comparison of vascular lesion frequency by sex, age at onset and duration

Sex and duration	Age at onset									All		
	0 – 14 (J)			15 – 39 (A)			40 – (L)					
	N	O	E	N	O	E	N	O	E	N	[illegible]	[illegible]
Males												
0– 4	133	1	0 5	386	2	4 3	944	115	124 8	1 463	118	129 6
5– 9	126	–	0 6	252	3	4 0	469	61	68 9	847	64	73 5
10–14	121	–	0 7	173	3	3 9	212	37	33 4	506	40	38 0
15–19	87	3	0 8	124	5	3 9	101	27	18 7	312	35	23 4
20–24	48	1	0 8	71	5	3 2	37	13	7 6	156	19	11 5
0–24	515	5	3 3	1 006	18	19 3	1 763	253	253 4	3 284	276	*276 0*
Females												
0– 4	125	–	0 0	251	2	6 0	1 423	246	239 6	1 799	248	245 6
5– 9	115	–	0 0	186	2	5 5	782	141	142 0	1 083	143	147 5
10–14	114	2	1 0	131	7	5 7	340	65	65 7	585	74	72 4
15–19	68	2	1.2	[illegible]	7	5 0	142	22	29 9	298	31	36 1
20–24	37	2	1 1	[illegible]	9	4 1	33	7	7.2	128	18	12 4
0–24	459	6	3 3	714	27	26 3	2 720	481	484 4	3 893	514	*514 0*

N = Number of registrations (duration cards)
O = Observed number of patients with vascular lesion registered in the duration period
E = Expected number calculated on the basis of observed frequencies by sex and age (Table 77)

Other diseases

As mentioned in Chapter II (p 44) the material for the clinical study of DM contains extracts from the case records about entries concerning the occurrence of other diseases A brief account of the data in respect of thyrotoxicosis allergic diseases tumours and rheumatoid arthritis will be given in the following sections The data refer to the registered occurrence on one or more occasions during a given 5 year duration period of the diseases in question With regard to all these diseases the numbers registered are rather small, no clear differences were found between the four investigation hospitals and therefore the figures will be given for the series taken as a whole As already stated the total number of registrations (duration cards) is 3 398 for males and 3,987 for females (cf Table 7A p 49)

Thyrotoxicosis

There are 19 registrations of thyrotoxicosis among males and 96 among females, or 0 6 per cent and 2 4 per cent, respectively The magnitude of the percentages, as well as the higher prevalence among females than among males is in accordance with what is ordinarily found both in the general population and among diabetics

It has long been known that thyrotoxicosis is often accompanied by glycosuria Whether this glycosuria is to be regarded as a manifestation of DM is still an open question

Allergic diseases

There are 40 registrations of allergic diseases among males and 42 among females or 1 2 per cent and 1 1 per cent, respectively Here, allergic skin reactions to insulin are not included In the great majority of cases the disease is bronchial asthma The occurrence of allergic diseases is evenly distributed over the observation series without any clear dependence on age or the duration of DM

Helander (1958) studied the occurrence of DM among 3 151 patients with bronchial asthma and the occurrence of bronchial asthma among 3 236 patients with DM The patients had been treated during the period 1937–54 at the allergy department and two departments of internal medicine of the Sahlgrenska Sjukhuset in Gothenburg The overall prevalence of DM among patients with bronchial asthma was 1 14 per cent, and the overall prevalence of bronchial asthma among patients with DM was 1 10 per cent this latter prevalence was the same for DM patients below and above 60 years of age

Helander quotes a series of earlier investigations which with a few exceptions seem to indicate an antagonism between DM and bronchial asthma (or allergy) It has been suggested that this antagonism might be due to low blood glucose levels in asthmatics on account of accelerated insulin production On the basis of his own data, however Helander arrives at the conclusion that the incidence of bronchial asthma among diabetics and the incidence of DM among asthmatics correspond entirely to the incidence of the two conditions in the general population

Our figures are in agreement with those obtained by Helander

Summarizing his conclusions from the scrutiny of the individual case reports, Helander makes the following statements

(1) ACTH adrenocorticotrophic hormone —may induce or at any rate permanently aggravate DM

(2) The onset of bronchial asthma is sometimes accompanied by diminution of the symptoms of previously existing DM and *vice versa*

(3) In some patients with both bronchial asthma and DM the disorders alternate

(4) Insulin in small doses may aggravate bronchial asthma in occasional patients, both when DM is present and when it is not

Therefore according to Helander, it would seem that in some patients the two disorders are antagonistic but this antagonism does not as previously supposed influence the incidence of one disorder in patients having the other Instead it seems to involve certain hormonal functions

Tumours

In all there are 37 registrations of malignant tumours among males and 8[illegible] among females or 1 1 per cent and 2 1 per cent respectively (of the number of duration cards) Here it should be taken into account however that the occurrence of malignant tumours implies 'a bias in so far as (because of excess mortality) these patients have a lower probability than others of being included in the subsequent 5 year duration periods In the duration group 0–4 there are 19 registrations of malignant tumours among males and 40 among females or 1 3 and [illegible] 2 per cent respectively (of the number of patients with DM duration 0–4 years) The prevalence figures increase with age but otherwise they are independent of the age at onset of DM and the duration of the disease

Rheumatoid arthritis

There are 31 registrations of rheumatoid arthritis among males and 94 among

females, or 0 9 and 2 4 per cent, respectively The prevalence figures increase with age but otherwise they are independent of the age at onset of DM and the duration of the disease

According to Engel and Roberts (1966) the prevalence of rheumatoid arthritis in the general population of the U S A increases markedly with age from 0 3 per cent at ages 18–24 to about 6 per cent at ages 55–64 (males 4 per cent females 8 per cent) and about 19 per cent at ages 75–79 (males 14 per cent females 23 per cent) For the age groups 18–79 taken together the prevalence figures are 1 7 per cent for males and 4 6 per cent for females, counting only "classical and definite cases (but not those designated as probable) the figures are 0 5 and 1 4 per cent Taking into account the age distribution of the patients in our series it can be concluded that rheumatoid arthritis seems to occur as frequently among DM patients as it does in the general population

In a series of 34 patients with both DM and rheumatoid arthritis with average duration of DM 11 1 years and of rheumatoid arthritis 12 2 years Powell and Field (1964) found a very low incidence and a high regression rate of diabetic retinopathy [1] According to the authors this may be an influence of the one disease or the other or it may be due to the large doses of salicylates which these patients have been taking for many years or to some other factor

Pernicious anaemia

At Vänersborg (P) a special inventory was made over the period June 1 1931–June 1, 1958 with regard to patients treated in the department of internal medicine for pernicious anaemia (cf Sundberg & Gronberg 1960) In all 314 cases were registered of these, 21 or 6 7 per cent had shown clinically manifest DM Of the patients with both diagnoses, 8 were males and 13 were females The mean age at onset of pernicious anaemia was 61 2 years, and the mean age at onset of DM was 62 0 years For 4 males and 8 females, DM was not present at the onset of pernicious anaemia but developed later after an average interval of 6 8 years For one female the diseases were diagnosed simultaneously in 1958 (the patient had no subjective symptoms of DM) The remaining 4 males and 4 females had had DM for an average period of 8 2 years before the onset of pernicious anaemia

Of the 314 patients with pernicious anaemia about 40 per cent are males and 60 per cent are females [2] The registration of the occurrence of DM among these patients must have been practically complete

At admission because of pernicious anaemia or at the later point of time when DM appeared, the mean age of the 21 patients with both diagnoses was 65 1 years (males 61 5 years, females 67 4 years) As can be seen from Table 34 (p 117) the aggregate morbidity risk for

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[1] For 4 of the 34 patients retinopathy of mild degree was registered at the patient s last visit another 8 patients had on an earlier visit showed either a single exudate or a streak haemorrhage or a few microaneurysms but had no retinopathy on one or more subsequent visits including the last Of 9 patients with a DM duration of 15 years or more only one presented retinopathy

[2] In 1950 Mosbech (1951) registered a prevalence of pernicious anaema of 1 0 per thousand population in Odense County Denmark the prevalence was 0 8 per thousand among males and 1 2 per thousand among females

On the basis of the prevalence figures by age given by Mosbech the aggregate morbidity risk for pernicious anaemia up to age 80 may be estimated at about 0 4 per cent for males and about 0 6 per cent for females

DM up to age 65 is 4 1 per cent for males and 7 0 per cent for females

As is indicated by these figures alone, there is in fact good agreement between the observed number of diabetics among patients with pernicious anaemia and the expected number (calculated on the basis of the morbidity risks for DM in the general population as shown in Table 34)

In our DM series covering a total of 867 patients (408 males 459 females) at Vänersborg Hospital over the period 1931–57, the age distribution of the patients would lead us to expect at the most 10 instances of pernicious anaemia However, taking into account that the proportion of admissions to hospital must be considerably larger in respect of pernicious anaemia than in respect of DM, the registration of 20 cases with both diseases during this period should not be interpreted to indicate an association between them [1]

[1] In the previous paper (Sundberg & Grönberg 1960) it was concluded that there might be a common denominator for the two diseases although the series was not sufficiently large to permit definite conclusions in this respect In accordance with the conceptions then current concerning the prevalence of DM the occurrence of 21 DM cases among 314 patients with pernicious anaemia was considered to justify this statement

CHAPTER VIII

Rating of DM risks in life and health insurance in Sweden

General developments

Towards the end of the nineteenth century interest began to be shown in several countries in the insurance of substandard risks In 1898 a Scandinavian committee was set up to study statistical and other problems relating to this field In the early years of the present century there arose in various quarters the belief that a more advanced form of cooperation would favour a rapid and satisfactory solution Sweden was one of the countries where these views first led to concrete measures In 1915 it was decided that the Sverige Reinsurance Company should be the central organization for the insurance of substandard risks (cf Strom & Svensson 1965 Larsson 1965a)

The functions of the Sverige as regards the insurance of substandard risks were to be twofold viz to rate the risks and to reinsure them

For the purpose of rating the risks the Appraisal Council of the Sverige (*Centrala riskprövningsnamnden*) was formed

The reinsurance of substandard risks is based on the idea of concentrating the internal part of the whole business—rating of risks calculations of premiums surrender values premium reserves and extra bonus technical and statistical analyses etc —within the Sverige whereas all contacts with the policyholders are managed by the direct writing companies It was thought that the pooling of the business by means of compulsory reinsurance would make it easier to accept the hazardous substandard risks than if each company was alone responsible for its risks In addition it would be possible to avoid unsound competition and to gain a wider statistical experience more rapidly

The rating and the reinsurance with the Sverige did not include all substandard risks The slightly substandard risks which the companies had already been in the habit of granting continued in the main to be handled by them These risks are termed B risks while those substandard risks which are accepted by the Sverige are called C risks and the standard risks are termed A risks Risks which are not accepted by the Appraisal Council

may be accepted in accordance with the decisions of the Comtra ("Cooperation internationale pour les assurances sur la vie des risques aggraves")—D risks[1] Risks which are not accepted at all will here be denoted R risks

In 1938 draft proposals for general directives with regard to the rating of risks were presented (by the medical director of the Sverige Dr Folke Lindstedt) These "guidelines" formed the basis of the underwriting manual which is now followed by all Swedish life and long-term health insurance companies in respect of individual insurance The manual as it exists in its current version has been evolved as the result of a number of revisions often effected at intervals of two or three years

Already when the C-risk insurance of the Sverige was initiated, there was an effort to use data collected through statistical investigations in order to classify and rate the risks in a way that would result in extra premiums that would be as correct as possible However uncertainty in respect of the prognosis due to insufficient experience meant that the risk appraisal remained very strict for a long time

During the course of the years considerable alleviations of the appraisal have been effected thanks to the general improvement of the prognosis of most diseases that has been brought about by medical progress and raised standards of living. Another important factor in this connection is that there has taken place a change of opinion as to the aim of the risk appraisal More and more the predominant attitude has been that the rating should not be stricter than is required to guard the company against undue speculation With the aid of statistics about the frequency of substandard risks of different kinds it has been shown that the substandard risks are not of such financial importance that the achievement of this aim might be considered unfair from the point of view of the standard risks

Hitherto these views have mainly been applied in respect of B risks (slightly impaired risks) in individual insurance and in respect of group insurance As regards C risks (moderately and severely impaired risks) in individual insurance, the extra premiums are still adjusted so as to correspond to the increased risk, but nevertheless an important change has taken place Whereas earlier the view was that the substandard risk premium should include a safety margin of roughly the same *relative* magnitude as does the standard risk premium it is now considered appropriate that the substandard risk premium shall include a safety margin of the same *absolute* magnitude as does the standard risk premium. This change of attitude and the actual improvement in the prognosis for the majority of diseases have enabled considerable reductions of extra premiums to be made in the case of C risks also

It should be added that all extra profits for substandard risks are distributed to the policyholders in question as a supplement to the ordinary bonus

[1] From 1957 Prior to that, D risks were accepted in cooperation between the Sverige and the corresponding companies in Denmark (Dana) Finland (Varma) and Norway (Norske Folk) From 1966 the Sverige accepts all D risks with sums not exceeding 50 000 kronor "for its own"

Technical methods

For C risks, the Sverige at first applied supplements which can be interpreted as an approximation to percentage mortality increases but after some time pure percentage increases were adopted In 1945 a new system was introduced the extra premiums were based on an additive supplement to the force of mortality (T risks) or on a combination of an age supplement and a constant supplement to the force of mortality (H risks) In the latter case however, the relation between the two supplements was chosen in such a way that the result was approximately a percentage mortality increase (however not of the net risk premium but with certain loadings for expenses included)

During a long period it was usual to restrict the insurance term for substandard risks by fixing a maximum final age This form of limitation has been extensively used in the case of diseases where mortality at advanced ages has been regarded as especially high, for instance circulatory diseases Diabetics have been subject to particularly severe limitations in this respect, a maximum final age of 45 or 50 years has not been unusual This system has now been completely abandoned as from 1956 there are no restrictions concerning the insurance term for diabetics

In 1956 the percentage increases for diabetics were substituted by age supplements

In 1958 a new type of increase was introduced in respect of diabetics (S risks) Diabetics were then rated with premiums which were based on an assumed extra mortality of the same type as that used in the case of H risks, but with greater importance attached to the age supplement which gave the mortality curve a steeper appearance According to the rules of 1964 the constant supplement was fixed at 1 per thousand whereas the minimum age supplement was [illegible] years and the maximum one—used if the onset of DM had occurred before 10 years of age—was 20 years When the age at onset was 40 years or over and other findings were normal, the rating was H1 (age supplement 5 years constant supplement 1 per thousand)

Partly on the basis of preliminary results from the present study a considerable liberalization of the appraisal of DM risks was introduced in 1966 The S risk rating was abandoned and H risk supplements were applied in respect of diabetics (cf below)

As from 1964 H risks are rated with permanent extra premiums which are based on an assumed extra mortality composed of an age supplement and a constant supplement as follows

Rating	Life insurance: Age supplement years	Life insurance: Constant supplement per thousand	Waiver of premium per cent	Health insurance (for DM) per cent
H1	5	1	50	50
H2	8	2	100	75
H3	11	3	150	100
H4	13	4	[illegible]	R

Premiums for standard risks

There have been several reductions in the mortality tables for standard risks Therefore if the rules for rating substandard risks had been kept unchanged,

the introduction of a new mortality table would as a rule have caused considerable alleviation of the supplements for sub standard risks

The mortality tables applied in indi vidual life insurance for standard risks (A risks) are shown in Table 79

Table 79 Assumed mortality for standard risks according to life tables D28, D37 D55 and M64

Age	D28 M F	D37 M F	D55 M F	M64 M	M64 F
	Force of mortality per thousand according to life tabl				
15	4 1	3 3	1 0	0 7	0 7
20	4 3	3 4	1 1	0 8	0 8
25	4 6	3 7	1 2	1 0	0 9
30	5 1	4 1	1 5	1 2	1 0
35	5 8	4 8	1 9	1 6	1 3
40	7 1	5 9	2 6	2 2	1 7
45	9 0	7 7	3 8	3 2	2 4
50	12 0	10 6	5 8	4 9	3 5
55	16 8	15 3	9 2	7 5	5 3
60	24 4	22 9	15 0	11 9	8 2
65	36 5	35 2	24 8	18 9	13 0
70	55 6	55 3	41 5	30 2	20 7
75	85 9	87 8	69 8	48 6	33 2
[illegible]	133 9	140 5	117 9	78 5	53 5
85	210 0	225 9	199 6	126 9	86 4
90	330 6	364 5	338 3	205 5	139 7

Rating of DM risks 1945-67

A brief account of the rating rules for DM in individual insurance from 1945 onwards is given in the following para graphs For the sake of brevity the older rules with regard to maximum final age are omitted It should be stressed that the rules are not applied mechanically but with due regard to the circumstances in the individual case this can be done without disadvantages because the rating is made by a central institution

Percentage supplements given in the tables refer to assumed force of mortality Thus 200 per cent means that the mortali ty at each age is estimated to be three times the assumed mortality at the same age among standard risks (with certain loadings for expenses included)

A *Manual of 1945*

(1) Within 6 months from diagnosis
postponement for [illegible] months

(2) Insulin treatment no complications

Age at application	*Appraisal for life insurance*
–19	R (reexamination at 20)
20–49	supplement 250–200 per cent
50–	supplement 200–150 per cent

all ages waiver of premium R

With long observation period certain alleviation

(3) Insulin treatment complications
R or increased supplement

(4) Dietetic treatment alone
as above with reduction 50–100 per cent

(5) Alimentary glycosuria with increased blood sugar level but without other dia betic symptoms
supplement 150–50 per cent

B *Manual of 1947*

(1) Within 6 months from diagnosis
postponement for 6 months

(2) Insulin treatment no complications

Age at application	*Appraisal for life insurance*
–14	[illegible]
15–19	at least 5 years of observa ion 250 per cent
20–29	250–200 per cent
30–49	200–150 per cent
50–	150– 75 per cent

With long observation period reduction 25 per cent

(3) Insulin treatment complications

R or increased supplement waiver of premium B

(4) Dietetic treatment alone long observation time

possibly some alleviation

(5) Alimentary glycosuria with increased blood sugar level but without diabetic 2 hour value and without other diabetic symptoms

75 per cent – normal

C *Manual of 1949*

(1) Within 6 months from diagnosis

postponement for [illegible] months

(2) Insulin treatment no complications

Age at application	*Appraisal for life insurance*
–14	R
15–19	at least 5 years of observation 250 per cent waiver of premium R
20–29	250–200 per cent waiver of premium R or increased supplement
30–49	200–150 per cent ditto
50–	150– 75 per cent ditto

DM with onset after age 50 is rated as milder than DM with earlier onset

(3) Insulin treatment complications

R or additional increase by 20–50 per cent

(4) Dietetic treatment alone long observation time

alleviation of condition stated above by 25–50 per cent

(5) Alimentary glycosuria with increased blood sugar level 200 mg per 100 cc or more but without diabetic 2 hour value and without other diabetic symptoms

75 per cent – normal

D *Manual of 1956*

Where glycosuria has been recorded once new urinalysis is asked for With more than one positive value glycose tolerance test is made

(1) Renal or alimentary glycosuria

normal

(2) DM Insurance granted not earlier than 6 months after the diagnosis Questionnaire to be filled up by the applicant If the sum exceeds 20 000 kronor, special certificate from physician required

The rules given below are applied with increased supplement where control has been insufficient or the applicant presents overweight or is prone to infections

(a) No vascular complications diabetic ocular changes or albuminuria

Age at application	Percentag increase to force of mortal ty for life insurance wi h duration of DM at appl ca tion (years)			
	5	6-10	11 0	21
–14	R	R	R	
15–24	300–250	D–300	D	R–D
25–34	250–150	300–250	D–300	R–D
35–44	150– 50	250–150	300–250	D–300
45–54	50	100– 50	250–150	300–200
55–	50	50	150– 50	200–100

At the calculation of premiums these percentage increases are substituted by age supplements (cf Table 80)

Waiver of premium 100–50 per cent where extra rating of life insurance does not exceed 200 per cent otherwise R

(b) Where vascular complications diabetic ocular changes or albuminuria are present

P

E *Manual of 1958*

Where glycosuria has been recorded once new urinalysis is asked for With more than one positive value glucose tolerance test is made

(1) Renal or alimentary glycosuria

normal

(2) DM Insurance granted not earlier than [illegible] months after the diagnosis Questionnaire to be filled up by the applicant

If the sum exceeds 20 000 kronor, special certificate from physician required

The rules given below are applied with increased age supplement 2–3 years where control has been insufficient or the applicant presents overweight or is prone to infections

(a) No vascular complications no diabetic ocular changes and no albuminuria

	Age at onset of DM				
	1 9	10-19	20- 9	30-39	40-
Life insurance	S4	S3	S2	S1	H1
Waiver of premium	R	R	150	100	50

(b) Where vascular complications diabetic ocular changes or albuminuria are present

R

Where ocular changes are very mild insurance may be granted possibly with extra age supplement 2–3 years

F *Manual of 1960*

As Manual of 1958 (E)

G *Manual of 1964*

As Manuals of 1958 (E) and 1960 (F) but with changed values for the supplements

Supplement	S1	S2	S3	S4
Manuals of 1958 (E) and 1960 (F)				
Age supplement years	8	13	17	22
Constant supplement per thousand	3 6	3 6	3 6	3 6
Manual of 1964 (G)				
Age supplement years	9	13	17	20
Constant supplement per thousand	1	1	1	1

H *Manual of 1966*

Where glycosuria has been recorded once, new urinalysis and test of fasting blood sugar are asked for With more than one positive value glucose tolerance test is made (if the sum is small fasting blood sugar value alone is considered sufficient)

(1) Renal or alimentary glycosuria

normal

(2) DM Life insurance (including waiver of premium) as a rule granted as soon as the diagnosis is made and the disease is under control health insurance granted not earlier than 6 months after the diagnosis Questionnaire to be filled up by the applicant If the sum exceeds 25 000 kronor (life insurance) or 300 kronor monthly (health insurance) special certificate from physician required

The supplements given below are applied

(a) No vascular ocular or renal symptoms

(a1) Good control without complications

Age at application	Age at onset of DM	Life insurance	Waiver of premium per cent	Health insurance per cent
–15		H4	R	R
16–30		H3	150	100
31–45	–39	H2	100	75
	40–	H1	50	50
46–	–54	H1	50	50
	55–	H0*	25	25

* H0 does not *per se* cause increased premium however with two different impairments H0 the rating is H1

(a2) Severe cases insufficient control overweight or proneness to infections in particular urinary infections

life insurance as a rule with increase one step in the table under (a1)—H0 to H1, H1 to H2 etc waiver of premium and health insurance as a rule R

(b) With vascular complications diabetic ocular changes or albuminuria

(b1) Ocular changes alone (retinopathy)

mild ocular changes (occasional microaneurysms) as (a),

proliferative changes R

other changes as a rule as (a2)

(b2) Albuminuria

intermittent findings with observation time at least 10 years as (a2)

otherwise as a rule R

(b3) Hypertension

appraisal as (a) and addition of separate supplements according to the rules applied in respect of hypertension

Remaining mean expectation of life

In order to illustrate the rating of DM risks Table 80 gives certain data about the remaining mean expectation of life at ages 20 30 40 50 and 60 for males The data refer to the rating of diabetics with a disease duration at application of 15 or 5 years according to the Manuals of 1956 1960 (=1958) 1964 and 1966 It is assumed that no complications have been registered or in other words that the rating is the most liberal one For comparison are shown the corresponding expectations according to the life tables

Table 80 Rating of uncomplicated DM risks in individual life insurance from 1956 onwards, and calculated mean expectation of life Males

Manual Life table		Duration of DM	Age at application 20	30	40	50	60
Rating							
DM	1956	15	+29 yrs	+23 yrs	+17 yrs	+12 yrs	+8 yrs.
		5	+27 yrs	+17 yrs	+9 yrs	H1	H1
	1960	15	S4	S3	S2	S1	H1
		5	S3	S2	S1	H1	H1
	1964	15	S4	S3	S2	S1	H1
		5	S3	S2	S1	H1	H1
	1966	15	H3	H3	H2	H1	H1
		5	H3	H3	H2	H1	H0
Remaining mean expectation of life years							
DM	1956	15	25 8	22 4	19 1	15 4	11 3
		5	27 5	27 5	25 8	21 0	13 7
	1960	15	30 0	26 0	21 3	17 6	13 7
		5	34 0	29 2	25 2	21 0	13 7
	1964	15	35 9	29 8	24 7	20 0	15 6
		5	38 5	33 2	28 1	23 1	15 6
	1966	15	41 9	33 6	28 4	23 1	15 6
		5	41 9	33 6	28 4	23 1	19 4
Standard risks	D55		52 4	43 0	33 7	24 9	16 8
	M64		55 4	45 9	36 6	27 7	19 4
Population	1941–45		51 2	42 6	33 6	25 0	17 2
	1946–50		52 1	43 0	33 8	25 0	17 1
	1951–55		53 1	43 7	34 4	25 4	17 4
	1956–60		53 5	44 0	34 7	25 6	17 5
	1961–65		53 6	44 1	34 7	25 6	17.5

Table 81 Mean expectation of life in the general Swedish population 1961-65, according to life table M64 for standard risks and with ratings H1 H2 H3 and H4 Males and females

Age	Remaining mean expectation of life years											
	Population 1961		M64		H1		H2		H3		H4	
	M	F	M	F	M	F	M	F	M	F	M	F
20	53 6	57 2	55 4	59 3	49 3	52 9	45 4	48 9	41 9	45 1	39 3	42 5
25	48 9	52 3	50 7	54 5	44 8	48 4	41 2	44 6	37 8	41 0	35 4	38 5
30	44 1	47 4	45 9	49 7	40 3	43 9	36 9	40 3	33 6	36 9	31 4	34 6
35	39 4	42 6	41 2	45 0	35 9	39 4	32 6	36 0	29 6	32 8	27 5	30 6
40	34 7	37 8	36 6	40 3	31 5	35 0	28 4	31 8	25 6	28 8	23 7	26 8
45	30 1	33 1	32 1	35 7	27 2	30 6	24 4	27 6	21 7	24 8	20 0	22 9
50	25 6	28 6	27 7	31 2	23 1	26 4	20 5	23 6	18 1	21 0	16 5	19 3
55	21 4	24 1	23 4	26 8	19 2	22 3	16 8	19 7	14 6	17 4	13 2	15 8
60	17 5	19 8	19 4	22 6	15 6	18 5	13 5	16 1	11 6	14 0	10 4	12 6
65	13 9	15 8	15 8	18 7	12 3	14 9	10 5	12 9	8 8	11 0	7 8	9 8
70	10 7	12 1	12 4	15 1	9 5	11 7	7 9	10 0	6 6	8 4	5 8	7 4
75	8 0	9 0	9 5	11 8	7 1	9 0	5 8	7 5	4 7	6 2	4 1	5 4
80	5 8	6 4	7 1	9 0	5 1	6 6	4 1	5 4	3 3	4 4	2 8	3 8
85	4 2	4 6	5 1	6 7	3 6	4 8	2 8	3 8	2 2	3 0	1 9	2 6
90	2 9	3 3	3 6	4 8	2 4	3 3	1 9	2 6	1 5	2 0	1 2	1 7

applied for standard risks in individual life insurance (D55 from September 1955 to August 1964 M64 from September 1964) and according to life tables for the general Swedish population from 1941–45 to 1961–65

In Table 81 are shown the mean expectation of life in the general population 1961–65 and according to life table M64 the data are given separately for males and females concerning standard risks as well as with ratings H1 H2 H3 and H4

Group life insurance

Group life insurance was introduced in Sweden in 1948 In view of the rapid developments which have taken place both with regard to the number of insured persons and with regard to the size of the coverage the rules for obtaining group life insurance and the practice applied for the risk appraisal are of great interest in the present connection A brief and somewhat simplified account is given below as a general fact it can be stated that nowadays the great majority of the diabetics in the active age groups have substantial amounts of group life protection and further, that the great majority of the juvenile diabetics become insured when they enter the labour market Certain restrictions exist as regards eligibility for insurance (cf below) but there are no special limitations on the validity of the insurance, and no extra premiums are charged for substandard risks

The type of group life insurance first introduced—*regular group life insurance*—is granted to groups of persons who are employed by the same employer or belong to a certain organization (instituted to take care of the interests of the members in their capacity of economically active persons) In addition this type of group life insurance has been extended to cover students organ

izations As a rule a group must comprise at least 25 persons and at least 75 per cent of those entitled to insurance

In 1961 there was introduced by collective agreements between associations of private employers and white-collar trade unions, another type of group life insurance—*occupational group life insurance*—which for the members of the employers association gives coverage to their employees (irrespective of whether they are members of the union or not) From 1963 similar arrangements have been made for blue-collar employees (workers) in private industry, and for all state and municipal employees

To a great extent regular group life insurance and occupational group life insurance for private employees include the wife (and nowadays also the husband) of the insured member From 1965 (and 1967) the same applies to the other categories of occupational group life insurance

For access to occupational group life insurance the only condition is that there shall exist an employment with the state a local government authority or an employer who is a member of an employers organization Hence there are no restrictions concerning DM or other diseases unless the state of health has been an obstacle to employment Nor are there any restrictions attached to the state of health of the spouse The basic amounts insured are 31 500 kronor for the employed person (up to age 55 with a gradual reduction to 4 000 kronor in the age interval 65–67) and 2 000 kronor for the spouse of an insured person (if there are children below 17 years of age) Including supplementary amounts to be paid if there are children below age 21 (9 000 and 4 500 kronor respectively for each child below age 17 gradual reduction for children aged 17–21) the average amount per employed person is about 44 500 kronor

In regular group life insurance the sums insured vary from comparatively small amounts up to 100 000 kronor or over but they are always fixed according to general rules (for instance the same sum for all members of the group or sums in proportion to the annual earnings) Generally there is also insurance for the wife (and sometimes for the husband) of the insured person The average amount per person (group members spouses) is about 20 000 kronor

Provided that the adhesion to a regular group life insurance is obligatory or the group is very large and entry takes place without delay the same rules are in the main applied in respect of the eligibility for insurance as in occupational group life insurance In the event of optional joining of the group or entry after delay certain conditions with regard to the applicant s state of health are to be fulfilled For a new group or for a general increase of the sums insured in an existing group it is sufficient that the applicant can establish that he is wholly capable of doing work and that during the preceding 12 months he has not been ill for periods longer than 14 days in unbroken succession these rules are used if the applicant is below 60 years of age and the amount does not exceed 60 000 kronor In other cases a questionnaire (health declaration) is to be filled up and the appraisal is made according to rules similar to those given in the Manual for individual insurance However in respect of DM standard rating is substituted not only for H0 but also for H1 and H2 If the insurance applied for cannot be granted with standard rating according to these lines insurance up to a total sum of 60 000 kronor may be accepted or entry may be postponed or refused (and the applicant can then have recourse to individual insurance)

When a person carrying group life insurance leaves the group (for instance because the group contract expires because the insured person transfers to other work without getting a group insurance or in respect of spouses because of the death of the group member or of divorce) there is a right to take out individual insurance corresponding to the expired coverage or the reduction in coverage without any conditions with regard to health examination

At the end of 1960 the total amount of group life insurance in force was 11 1 billion (thousand million) kronor as compared with

a total amount of 22 4 billion kronor individual life insurance

At the end of 1967 the total amounts of group life insurance and individual life insurance may be estimated at about 150 billion kronor and about 30 billion kronor, respectively Of the first mentioned sum about 35 billion kronor relate to regular group life insurance and 115 billion kronor to occupational group life insurance (including the group life protection for state employees which is given in accordance with a special law and not in the form of an ordinary insurance policy)

Group health insurance

With regard to health insurance it should be kept in mind that the Swedish social security system includes both invalidity pensions and health insurance on a compulsory basis (cf p 19) [1] Nevertheless group health insurance is developing rapidly In this field the particular problems of risk appraisal have been solved by means of a clause which restricts the payments under the insurance in cases of working incapacity caused by disease (accident) that has occurred before the issue of the policy The payments under the policy (including stipulated waiting period) are limited to cover at the most a period equal to the time that has elapsed since the latest date before the issue of the policy at which the disease (accident) or sequelae thereof caused incapacity or treatment (prescription) was given by a physician deduction is made for periods after the issue of the policy during which the disease (accident) or sequelae thereof have caused working incapacity

If for example the policyholder was treated by a doctor six months before the issue of the policy and was thereafter capable of doing work but falls ill when the policy has been in force for three years he will be entitled to payments for a maximum period of three and a half years If his incapacity lasts for two years he will after a further period of one year be entitled to payments for a maximum period of two and a half years (3 5−2+1)

The clause may be excluded if the applicant gives a satisfactory health declaration which is accepted by the company

Present situation and further considerations

DM is a common disease As can be seen from Table 35, the life table expectancy for DM up to age 70 is about 4 per cent for males and about 8 per cent for females The number of diabetics in Sweden below age 70 can be estimated at about 110 000 (cf Table 32)

Social and economic developments have meant that nowadays diabetics who possess working capacity are employed under the same conditions as non diabetics The mobility of the population has greatly increased both with regard to change of residence and with regard to change of type of activity persons move from the countryside to the large towns and other agglomerations self employed persons become employees and

[1] *National Insurance Act* promulgated at the Royal Palace in Stockholm May 25 1962 Revised translation July 1966 Swedish Ministry of Health and Social Affairs Esselte AB 1966 pp 1–56 — The booklet contains the text of the National Insurance Act with amendments having come into force not later than January 1 1967

employees become "self employed" Very often these changes lead to an interest in—or a need for—higher insurance protection Similar effects arise as a result of increasing costs of living as well as increasing real income

As will have appeared from the preceding sections the rating of DM risks in individual insurance has been largely liberalized especially in the revisions of the Manual that were undertaken in 1964 and 1966 The rapid growth of group life and group health insurance have largely contributed to the effect that diabetics nowadays generally have considerable insurance protection as belonging to groups for which the participation is secured through agreements between the parties of the labour market through legislation (for state employees) or through membership of an organization of "self-employed persons a students organization or the like No extra premiums are charged for substandard risks in these types of insurance Probably in Sweden the average diabetic has a higher life and health insurance coverage and more liberal insurance conditions than is the case in most other countries

However the interest for coverage in the fields of life and health insurance is to a great extent connected not with the absolute needs alone but rather with the relative needs', viz the comparison with other persons in a similar economic situation Therefore it may be said that neither the extension of the insurance coverage under the Swedish social security system nor the developments within group life and group health insurance have eliminated the problems connected with the granting of individual insurance to persons with an impaired state of health

There is a demand that people shall as far as possible be eligible for life and health insurance protection not only at younger ages but also at comparatively high working ages

Against the background of these needs and demands it must be considered highly desirable that the appraisal rules applied by the life and health insurance companies should be made very liberal It may even be maintained that extra premiums should be charged only in order to guard the corps of policyholders as a whole against losses from *undue* speculation the primary goal should be not to apply appropriate extra premiums to persons who are substandard risks, but solely to prevent them from taking out higher insurance protection than they would have done if their state of health had been normal Where measures against such a counterselection are needed in the interest of the whole body of policyholders it will as a rule be sufficient to apply comparatively low extra premiums preferably over the initial period of the insurance term

A secondary question is to what extent the risk appraisal should be taken into account in respect of the distribution of bonus to different categories of policyholders The goal should be to arrive at a proper balance between strict equity and solidarity which enables simple and consistent solutions to be arrived at

The prognosis of DM is heterogeneous medical experts are of opinion that it is extremely difficult to make an individual prognosis even for diabetics who have been the object of thorough clinical

examinations over a long period The uncertainty is still greater in respect of new cases Nevertheless, provided after a number of observation years the disease has not become complicated by nephropathy or severe ocular or vascular changes there seems nowadays to be general agreement that *on average* the prognosis is in the main favourable In the age interval 55–69 the excess mortality of diabetics cannot reasonably be assumed to exceed 30 per cent, and at higher ages their excess mortality is still lower (less than 20 per cent)

In respect of life insurance the most serious complication in DM is the occurrence of nephropathy There seems to be unanimity among experts that as a rule diabetics with clear nephropathy should not be granted individual life insurance The occurrence of retinopathy is not *per se* considered an obstacle to life insurance probably the excess mortality for diabetics with retinopathy—without the combination with nephropathy—is slight From the point of view of health insurance on the other hand due account must be taken of the risks of seriously impaired visual acuity

Hypertension does not seem to be a greater risk in otherwise healthy diabetics than in non-diabetics

On the basis of these considerations one of the present authors (Larsson chairman of the Board Delegation of the Sverige) has suggested that new rules for the appraisal of DM risks in individual insurance should be tried on the following lines

(a) Cardiovascular changes should be rated according to ordinary rules

(b) Loss of visual acuity should be rated according to ordinary rules

(c) Where ocular changes beyond aneurysms and slight exudates are present there should be applied in respect of waiver of premium and health insurance a clause for loss of visual acuity or a comparatively high extra premium, otherwise, the rating should be made without regard to the ocular changes

(d) Where the occurrence of permanent or recurrent albuminuria has been established, the application should be refused, or possibly a special type of policy with "graded limitation (gradually increasing insurance sum up to the full amount after a suitable number of years) might be issued, in the presence of nephropathy, the special circumstances in the individual case should be taken into proper account (the sum insured, the applicant s reason for seeking insurance occupation degree of control etc)

(e) Apart from the restrictions stated above the premiums for diabetics should be based on supplements, which would be gradually reduced according to the age at onset of DM and the duration of the disease until H0 for age at onset above 40 and duration above 5 years for age at onset 30–39 and duration above 10 years for age at onset 20–29 and duration above 15 years, and for lower age at onset and duration above 20 years Reduction of the higher supplements should be granted automatically or, possibly, after verifying that nephropathy has not developed

(f) Possibly a premium system of this kind should be combined with altered rules for the distribution of bonus for instance an appropriate allocation of the

extra bonus among policyholders who have reached the H0 class[1]

This proposal—and several others—were thoroughly discussed at the 1966 revision of the Manual but at that time the statistical analysis presented in Chapter IV, was not sufficiently complete and it was considered preferable to undertake a liberalization more in accordance with traditional lines

It is our hope that the present study will be of use to the medical directors actuaries and other experts of life and health insurance companies in the further discussion of a revision of the appraisal rules in respect of DM

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[1] Such a method for the allocation of bonus cannot be regarded as inconsistent with the principle of equity since it is equivalent to a system where the extra bonus is continually invested as single premium for a pure capital insurance (to be paid in the event of survival but not in the case of death)

CHAPTER IX

Genetics of DM

Current conceptions and discussions

There are many reports concerning familial occurrence of DM and the possible significance of genetic factors for the aetiology of the disease Various genetic mechanisms have been considered such as recessiveness or dominance of an autosomal major gene (with incomplete or irregular penetrance) and multifactorial inheritance It has also been suggested that an aberrant major gene may cause juvenile DM in homozygotes whereas the milder maturity onset DM develops in heterozygotes

However so far as we have been able to find the great majority of these reports are based on observations (or hypotheses) of the morbidity risks for DM that lie far below the figures found by us Further the sex differences with regard to the morbidity risks (at ages above 50) are generally assumed to be comparatively small As an example the following series of prevalence rates may be quoted it refers to estimates concerning the frequency of recognized DM which are based on household interviews of the civilian *non institutionalized population in 1,000 sampling units throughout the* U S A [1] For comparison the corresponding figures from our assessment in Chapter IV are shown [2]

Attained age	Prevalence of DM per thousand population			
	U S National Health Survey 1957–59		Swedish mortality statistics 1961–63	
	M	F	M	F
0–24	1 1	0 7	(2)	(2)
25–44	4 9	3 8	11	11
45–54	11 2	13 7	21	19
55–64	25 2	31 5	32	49
65–74	34 4	50 3	45	87
75–	31 5	38 8	54	99

* Primary and contributory causes of death corrected for gaps excess mortality according to Assumption II cf Tables 32 and 34

According to *Fritz Lenz (1923)* it seems likely that in some families the mode of *inheritance is dominant* but that there are also recessive genes which may condition the occurrence of DM In spite of the higher prevalence among males than among females Lenz does not consider a sex linked transmission to be probable [3]

[1] U S National Health Survey 1960 Health Statistics *Diabetes reported in interviews United States July 1957–June 1959* Public Health Service Publication No 584-B21 pp 1–22

[2] The age distribution (within the age groups) is not the same in the two series but considering the large differences this is of minor importance only

[3] Lenz states that the frequency of DM among males is more than twice as high as among females.

Cammidge (1928 1934) studied 1 000 DM cases and found a family history of the disease in 396 of these He tried to classify the pedigrees according to whether they were the product of recessive or dominant inheritance and arrived at the conclusion that the recessive type developed earlier in life than the dominant type Thus early onset DM might in general be due to homozygosis for a recessive gene whereas late-onset DM should probably be regarded as being caused by a (different) dominant gene

Then Bergh (1938 1939) investigated the state of health of twins in monozygotic co twins of DM patients she found a much higher concordance rate than in dizygotic co twins

Hanhart (1939 1947 1950 1951 1953) made a series of thorough family investigations concerning the heredity of DM His results are considered to speak decidedly in favour of the theory of a recessive mode of inheritance

Penrose and Watson (1945) presented data indicating the greater incidence of DM in like sexed sibs They seem to be of opinion that DM is primarily conditioned by an autosomal dominant gene but that there exists in addition a secondary modifying gene which is sex linked (recessive) Their interesting paper will be discussed later (p 229)

In a study of a considerably larger series which included part of the sample studied by Penrose and Watson Thompson and Watson (1952) failed to confirm the finding of a sex linked tendency the authors conclude that their data are suggestive of inheritance by a single autosomal recessive gene

At the request of a Swedish governmental committee (cf p 114) von Hofsten (1948) surveyed the current views concerning the heredity of DM and connected problems His discussion is mainly hypothetical but is nevertheless of great interest in the present connection

In von Hofsten s opinion it is proved that to a great extent probably in the majority of cases DM is inherited. There are families in which the only way of interpreting the data seems to be the acceptance of mono hybrid dominant transmission On the other hand it seems possible that the most frequent form of DM is inherited as a monohybrid recessive trait but there are alternative explanations which might be equally probable The disease may be conditioned by a dominant gene which is manifested only in the case of constitutional weakness of one kind or the other or in the presence of certain environmental influence Further it is not proved that the apparently recessive form of DM is unitary it is possible and not unlikely that there exist at least two different types of recessive DM

On the basis of assumptions concerning the frequency of the diabetic genotype in the general population and concerning the fertility of diabetics von Hofsten discusses the implications of a reduced antiselection against the aberrant gene (due to reduced excess mortality and increased relative fertility among diabetics)[1] Whichever assumptions are made with a prevalence of the DM genotype ranging from 0 3 per cent to 4 per cent and a reproduction of the DM genotype between 90 and 75 per cent of that of the general population von Hofsten arrives at what he considers un acceptable values for the mutation rate (from normal to diabetic gene) Therefore the suspicion cannot be avoided that to a greater extent than geneticists generally assume DM is not hereditary or is as cribable to non specific genes several other genes with different action might be expressed in the form of DM also or might entail a certain disposition for the development of DM

In his discussion with regard to eugenic

[1] It is worth noting that according to von Hofsten a value of 0 6 per cent for the frequency of the DM genotype in the general population appears *a priori* very high In another connection he says If the frequency is assumed to be so unreasonably high as 4 per cent

measures von Hofsten is opposed to sterilization but he advises against marriages between diabetics and further states that a diabetic should hardly marry a person whose father or mother or sibs have DM Fertile matings between two diabetics should always be regarded as unsuitable However, he emphasizes that it is improper, not to say irresponsible to arouse apprehensions which are based solely on uncertain suppositions For instance it is anything but desirable that healthy persons should be given the impression that there exists a probability of 50 or 25 per cent of their developing DM or that healthy parents without any known instances of DM in their families should, after having got a child with DM, have to be worried that their other children will develop the disease

Harris (1949, 1950) underlines that data from hospital admissions tend to underestimate the relative frequency of the late onset cases in the general population and overestimate the relative frequency of the juvenile cases Juvenile cases are generally severe and are all or nearly all sent to hospital clinics for investigation or treatment Late onset cases are very often quite mild and many of them probably never find their way at any time to a hospital clinic An exact age at onset distribution could be arrived at only by a very elaborate population survey

On the basis of detailed family data for a series of 1 241 DM probands Harris concludes that there is a true positive sib-sib correlation with respect to the age at onset of DM This implies that the disease is heterogeneous the early-onset and late onset cases being determined by different gene combinations On the other hand it is apparent that mild cases of late onset occur not infrequently among the parents and other relatives of the severe juvenile and young adult forms of DM although there appears to be little or no parent-child correlation with respect to age at onset This evidence does hardly accord with the view that we are dealing with two genetically distinct and separate diseases Nor is it consistent with an explanation accounting for the variation in age at onset on the basis of autosomal modification

In the early onset cases there is evidence of an increase in parental consanguinity (greater than that in the general population) this observation suggests that many if not all of the juvenile and young adult cases are probably to be regarded as homozygotes for a single mutant gene The finding that no such rise in parental consanguinity in the late onset cases could be detected implies that these cases could not in the main be regarded as homozygous for the same gene

One hypothesis which might give a reasonable description of the observations is that many of the mild late onset cases could be regarded as heterozygous for a gene which in homozygous form gives rise to the severe early onset type of DM The distribution of the homozygous and heterozygous cases both in respect to age at onset and to severity would be presumed to overlap to a certain extent There would be incomplete manifestation particularly of the heterozygotes which would be dependent on environmental factors and genetic background However it is possible that the high sib sib and low parent-child correlations with respect to age at onset may in part at least be accounted for by modification due to common recessive autosomal modifying gene or genes or to allelic modifiers

The question whether we have to deal with a single gene pair or an allelic series remains open as also does the problem whether mutant genes at more than one locus may not be producing similar end results The exact conditions of manifestation and the influence of environmental factors will also require detailed study

The views expressed by Kemp (1951) in his well known textbook *Genetics and Disease* can be summarized as follows

The prevalence of DM is about 0 4 per cent in the U S A England Norway and Denmark The registered prevalence has been rising during the past 50 years or so owing to improved diagnostic possibilities

the increased longevity of the population in general and particularly of diabetics as well as the fact that diabetics can now have more children than previously The prevalence is highest (2–4 per cent) among women aged 60–79 The morbidity risk is 1–5 per cent for males and 2–7 per cent for females the average risk for the whole population has been calculated at about 6 per cent

It is probable that the majority of diabetic cases are hereditary and that there exist two or more genes which can produce DM There must be at least one gene for the juvenile form and one for the senile form but it is more reasonable to suppose that there exists a series of allelic diabetogenic genes Nor can we exclude the possibility that mutations in quite different places can give rise to disorders of the carbohydrate metabolism which it is impossible to distinguish from one another by clinical methods There seems however to be a genetic relationship between the different forms of DM

After having mentioned the theory advanced by Harris (cf p 216) that juvenile DM might be due to a gene present in the homozygous form and that the same gene in heterozygous form might produce senile DM Kemp continues The conditions are however hardly so simple Older theories to the effect that all cases of diabetes depend either on a single recessive gene or on a single irregularly dominant gene have now been abandoned

Joslin (1953) in his *Diabetic Manual for the Doctor and Patient* states without reservation that the tendency to diabetes is inherited as a recessive trait

Bartels (1953) on the basis of a review of the literature concludes that an estimate of potential diabetics at 6 per cent (of the American or Danish population) would not be too high If the assumption of recessive inheritance is correct for all diabetes of obscure aetiology the number of carriers of the gene in the population would be in the order of 25 per cent

Lamy Frezal and de Grouchy (1957) investigated the incidence of DM among relatives of 500 probands No correlation was found between maternal age or parity and the incidence of DM For the severe insulin treated diabetics whether juvenile or late there was found consanguineous marriages in the parents DM among both paternal and maternal relatives and an increased prevalence of DM among the sibs if one parent was diabetic The authors conclude that severe DM whether juvenile or late is due to the homozygous action of a gene which in the heterozygous condition leads to mild adult DM

Lamy Frezal and Rey (1961) adduced the supplementary theory that in addition to the main gene there exists a number of modifying genes which affect the expression of the major gene With a great many of these secondary genes present DM might occur even in the absence of the major gene

Grunnet (1957) made an extensive study of the prevalence of DM among relatives of DM probands Grunnet is of opinion that there exists a type of late-onset DM which is inherited as a dominant trait In addition there are other types of hereditary DM in which both mild cases with late onset and severe juvenile cases occur the fact that the frequency figures are higher for sibs than for parents gives rise to the conclusion that part of these cases are ascribable to homozygosity for a recessive gene or to the combined action of several genes

White (1959) seems to be firmly of opinion that the potentiality for developing DM is inherited as an autosomal recessive trait She supports her views primarily upon five facts

(1) the concordant occurrence of DM in similar twin mates

(2) the statistically greater frequency of DM in close blood relatives of diabetics than in those of control populations

(3) the demonstration of Mendelian ratios of the recessive type in large series of cases selected at random

(4) the demonstration of expected ratios in presumably latent (tested) cases

(5) the fact that the incidence of DM in genealogies of diabetics behaves as a recessive trait

Steinberg (1959) estimated that about 5 per cent of the population of the U S A is genetically liable to DM He emphasizes that in all sets of data that are presented so that comparisons can be made it is found that twice as many sibs of DM probands are diabetic if one parent is diabetic than if neither parent is diabetic dominant inheritance does not lead to such a pattern According to Steinberg susceptibility to DM is probably inherited as a recessive character this hypothesis is the only one consistent with the several large samples of family data that have been published It is not clear from the available data whether homozygosis at only one locus or at any one of two or more loci will cause susceptibility to DM

Widukind Lenz (1961) makes a series of interesting remarks concerning the difficulties of distinguishing between recessiveness and dominance in respect of common genes His starting point is that about 2–4 per cent of the population in Western Europe and the U S A become afflicted with DM (in general at fairly high ages) Twin studies have revealed that about 50 per cent of the co twins of monozygotic twins with DM do not become afflicted and Lenz therefore assumes that at least 8 per cent of the population carry the genetic disposition for DM and hence in the case of recessiveness the gene frequency would be 0 28 About 40 per cent of the population would be heterozygotes Then it would often happen that a homozygote (developing DM) marries a heterozygote in this case it is to be expected that 50 per cent of the children will be homozygotes and pseudodominant inheritance would not be an exception but a rather common situation

According to Clarke (1962) the most widely held view is that DM is inherited as an autosomal recessive trait but that the gene has only 20 per cent penetrance However, with regard to the statistics adduced by Pincus and White (1934) and by Steinberg and Wilder (1952) which show a good fit to the expected ratios Clarke makes the remark that, with a penetrance of only 20 per cent it is impossible to be sure that the genotype of the parents has, in fact been scored correctly

The alternative possibility is that diabetes is controlled multifactorially By analogy with other diseases the commonness of the condition makes this hypothesis likely Supporters of this view point out that there is no clear-cut division between normal and abnormal glucose tolerance tests It may be queried however, whether this test reflects the primary action of the gene

Clarke s conclusion is that since the matter is undecided there is a good case for further investigation (cf also Clarke 1961)

The data given by Nilsson (1962 1964) will be discussed in a later section (p 237)

Simpson (1964) made a thorough genetic analysis of family data for 2 645 probands from a register of diabetics in Canada (cf also Simpson 1962) The observed numbers of diabetics among sibs parents and children are compared with expectations for the general population (observations from three small towns in Ontario and a city in the Province of Prince Edward Island) According to the author her data suggest that the hereditary basis for DM is multifactorial [1]

Pfandler (1964) in his comprehensive review of the genetics of metabolic disorders, seems to favour the explanations put forward by Grunnet Pfandler states that many geneticists hold the view that family studies as well as statistical data speak in favour of simple recessiveness the same gene would be responsible for the juvenile

[1] It should be remarked however, that the expectations are calculated from prevalence figures that are lower than those found by us (cf p 111) for males the maximum prevalence applied is 3 90 per cent (at ages 70–79) and for females it is 4 47 per cent (at ages 60–69)

and the late forms of DM If there are instances of DM which are conditioned by dominant genes these are at least less frequent The occurrence of heterogeneity is not proved Against these theories stands the hypothesis adduced by Grunnet of a heterogenic and possibly polygenic causation of DM

On the other hand Arthur G Steinberg who has devoted several studies to the genetic problems of DM (Steinberg & Wilder 1952 Steinberg 1955 1958 1959 1961 1965) has presented data which do not fit the hypothesis of multifactorial inheritance

With regard to the theory that early-onset diabetes is due to the effects in homozygotes of a recessive gene which in heterozygotes leads to late-onset diabetes Steinberg (1965) reports several investigations concerning the incidence of diabetes in offspring which in his view reveal facts incompatible with this hypothesis In series published by Harris (1950) and by Steinberg and Wilder (1952) the frequency of diabetics among parents of patients becoming afflicted before age 30 was found to be 3 3 and 5 0 per cent respectively whereas among parents of patients becoming afflicted after age 30 the corresponding frequencies were 6 2 and 11 4 per cent Although—as Steinberg points out—in these series the parents of the late onset diabetics are on average older than those of the early-onset diabetics the deviations from expectation must be considered to argue against the theory—according to which the frequency of diabetes among the parents of patients with early onset should be greater than the frequency among the parents of patients with late onset A further argument against the theory was the fact that the prevalence of diabetes among the sibs was greater when one parent was diabetic than when neither parent was diabetic The data adduced by Steinberg are shown in the table below

On the basis of data from the literature concerning the prevalence of diabetes among the sibs of diabetic patients Steinberg makes a comparison between the situation where one parent is diabetic and the situation where neither parent is (known to be) diabetic The data cover a total of 3 923 and 21 774 sibs respectively Of these 11 4 and 4 4 per cent were diabetic the quotient between the percentages being 2 58 From this Steinberg concludes that a simple working hypothesis not necessarily correct but one which fits the data is that susceptibility to diabetes is due to homozygosis for a recessive gene

For the subsequent analysis it seems warrantable to give a quantitative example of the assumptions involved in the use of such a quotient as the basis for conclusions concerning the mode of inheritance of DM

With unbiased registration of the patients complete knowledge about the genotype of the parents the same average family size and the same age

Series	Patient's age at onset	Number of sibs, and frequency of diabetes among sibs of the patients, per cent					
		One parent diabetic		Neither parent diabetic		Total	
		No	%	No	%	No	
Harris 1950	0–29	48	18 8	971	3 5	1 019	4 1
	30–	327	10 7	2,446	4 1	2,773	4 4
Thompson & Watson 1952	0–29	57	15 8	425	6 4	482	7 5
	30–	714	15 3	3 411	7 8	4 125	9 1
Steinberg & Wilder 1952	0–29	92	14 1	736	5 0	828	6 0
	30–	1 528	11 2	5 928	4 6	7 456	6 0

Prevalence of the diabetic genotype r^2	*Prevalence* of the diabetic gene r	*Prevalence of* diabetic phenotype among parents with diabetic genotype		Ratio between the prevalence of diabetic genotype in sibs with diabetic parent and that in sibs with one other parent diabetic	
		(1)	(2)	(1)	(2)
0 02	0 1414	0 58	0 56	2 95	2 86
0 03	0 1732	0 48	0 46	3 05	2 93
0 04	0 2000	0 41	0 40	3 13	3 04
0 07	0 2646	0 31	0 30	3 33	3 24
0 10	0 3162	0 26	0 25	3 48	3 40
0 13	0 3606	0 23	0 22	3 62	3 54

distribution for the sibs the expected value of the quotient would be 2 0 If in addition, there had been complete knowledge about the genotype of the sibs (and they had been classified accordingly) the frequencies of diabetics among them would have been 50 and 25 per cent (instead of 11 4 and 4 4 per cent)

Let us for simplicity assume that of parents with diabetic genotype the fraction x is included in the diabetic phenotype A (= affected) and the fraction $y = 1 - x$ is included in the non diabetic phenotype U (= unaffected) which with complete ascertainment of their state of health will imply that they have reached an age corresponding to $100x$ *per cent* of the total morbidity risk Let us further assume that the conditions in respect of unbiased registration and the same average family size are fulfilled Denoting the prevalence of the recessive gene by r and the prevalence of the allelic "normal" gene by $d = 1 - r$ we get the distributions by type of mating shown in the following table

Phenotype of mating	Frequency of mating stated in the general population	Distribution by type of parental mating of sibs of diabetic probands
A × A	$r^2 \ x^2$	$4r^2 \ x^2 (1+r)^2$
A × U	$2rx \ (d \ \ ry)$	$4rx \ (d+2ry) (1+r)^2$
U × U	$(d+ry)^2$	$(d+2ry)^2 (1+r)^2$
Total	1	1

With the assumptions made, the number of sibs can be utilized for determining x We have a total of 25 697 sibs of whom 21 774 are in families with neither parent phenotypically diabetic and 3,923 in families with one parent diabetic, for illustration we can take this latter figure to represent (1) either only *one* parent diabetic or (2) *one or both* parents diabetic

With different values r^2 for the prevalence of the diabetic genotype in the general population we get the frequencies given at the top of this page

As will be seen from this table the ratio 2 58 found by Steinberg must be regarded as *low*, although the theoretical value with unbiased registration and complete knowledge of genotypes is 2 0 in (1) and somewhat less or $2(4-4r)/(4-3r)$ in (2) An interesting feature is that the ratios show a comparatively *small variation with the gene frequency r*

At a symposium on the genetics and epidemiology of chronic diseases in 1963 an interesting paper concerning the evaluation of genetic factors in DM by Neel Fajans Conn and Davidson (1965) was *presented The authors summarize their* results as follows

The data obtained once again confirm the familial nature of diabetes but

in the strict and formal sense are not proof of a genetic predisposition although by exclusion this seems highly probable It is shown that in the diabetically predisposed (offspring of conjugal diabetics) there are highly significant changes in the mean glucose tolerance curve in the 10–29 age interval This fact can be utilized in the analysis of family data Assuming a genetic basis then the results of our analyses do not favor the hypothesis that diabetes is due to homozygosity for a single recessive gene unless one is willing to assign a frequency of 0 20–0 25 to the recessive genotype There appear to be valid reasons for discarding other simple genetic hypotheses The data are consistent with a multigenic (multifactorial) hypothesis although the apparently very high proportion of affected offspring from conjugal diabetics remains a troublesome point This is explicable if the living conditions of the current generation have lowered the threshold of phenotypic expression of the diabetic genotype below that of the paternal *generation*

The data adduced by Neel Fajans Conn and Davidson are based in part on the results of glucose tolerance tests of individuals in a community in Michigan The values of the tests did not show a bimodal distribution Similarly the tests of the offspring of families with a diabetic child and at least one normal parent did not show bimodality Were predisposition to diabetes determined by a simple genetic mechanism—and reflected by an abnormal glucose tolerance test—a bimodal distribution would be expected A further argument for the theory of multifactorial heredity was that the test data indicated that there were too many diabetic offspring in these families for predisposition to diabetes to be due to recessive inheritance

In this latter respect Steinberg (1965) objects that while it may be true that all diabetics have abnormal glucose tolerance tests it is probably not true that all those with abnormal glucose tolerance tests are diabetic or prediabetic The thorough study made by Neel Fajans Conn and Davidson gives valuable knowledge concerning the variability of the outcome of glucose tolerance tests However although glucose tolerance tests are useful in the diagnosing of DM they are not the diagnosis [1]

Jorgensen (1966) underlines the difficulties in the genetic analysis of DM connected with the high prevalence of the disease the great variability of the disease picture and the strong age dependence of the clinical manifestation the age corrections which are necessary for numerical calculations are complicated and in addition very uncertain

Jörgensen does not exclude the possibility that sometimes there may be found special genetic types of DM with monohybrid transmission but considers the great majority of cases of DM to be due to a multifactorial system of additive genes with threshold effects

(1) The variability of blood sugar level with unnoticeable and unclear transition from still healthy conditions via prediabetic states to clinically manifest DM is adduced as one argument in general monohybrid genetic diseases show far less variability than do polygenic diseases

(2) A second argument is the frequent

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[1] Certainly the theory that subnormal intellectual capacity—as indicated by an IQ below say 100 per cent or below 90 or 80—is conditioned by an aberrant major gene can be disproved by a similar type of analysis In the main intellectual capacity is a graded character to a great extent connected with multifactorial inheritance However as is well known there are many forms of oligophrenia which are conditioned by single gene mutations (cf for instance Sjogren & Larsson 1949 1957 1967) or by chromosome aberrations

Height is a graded character with clear elements of multifactorial inheritance this does not preclude among persons of short stature the occurrence of instances of dwarfism which is conditioned by a major gene

Although senility may be mainly connected with multifactorial inheritance and exogenous factors essential senile dementia is conditioned by a major gene (Larsson Sjogren & Jacobson 1963)

occurrence of isolated cases but on the other hand a clearly increased morbidity risk among relatives as compared with that of the general population

(3) A third argument is that there is good agreement between the morbidity risks for parents and sibs and further that the morbidity risks among more distant relatives decrease rapidly (which does not occur to the same extent in the case of monohybrid dominance)

(4) A fourth argument is given by reference to the results of twin research Jorgensen quotes data from the literature according to which the concordance rate for monozygotic twins is more than four times the concordance rate for dizygotic twins (93/151 or 62 per cent and 43/334 or 13 per cent respectively)

(5) Finally it is stated that a series of investigations have shown that there is an increased frequency of DM among persons with blood group A Jorgensen considers it possible that the A blood group gene is the first of the factors to be identified in the multifactorial genetic system of DM (and several other multifactorially conditioned diseases)

In connection with these five points it will merely be commented here that not only diseases with multifactorial inheritance but also diseases conditioned by a dominant major gene may show great variability with regard to age at onset symptoms and course There are different opinions among experts about the possibility of an association between DM and blood group A The statistical results in respect of the frequency of isolated cases the concordance between twins and the morbidity risks among relatives will depend on the magnitude of the morbidity risk for DM in the general population and the shape of the morbidity risk curve These questions will be further discussed in a later section

Pavel and Pieptea (1966) investigated the occurrence of DM in three or four successive generations in the families of diabetics registered with the Antidiabetic Centre of Bucharest Among 12 000 patients there were 3 430 cases which are interpreted as being hereditary (the remaining isolated cases are said to be des cas de diabete acquis) Excluding 42 pedigrees with DM on both the paternal and the maternal side and 4 pedigrees containing consanguineous marriages there remained for analysis 113 pedigrees with DM consecutively over three generations and 4 pedigrees with DM consecutively over four generations The number of affected persons in the different generations is as follows

Number of diabetics in Generation				Number of pedigrees
C	P	GP	GGP	
2	7	2	1	1
1	5	2	1	1
1	3	1	1	1
1	2	3	1	1
4	1	1	–	1
3	1	1	–	4
2	1	1	–	19
1	2	1	–	6
1	1	1	–	83
		Total		117

C = children P = parent (and sibs of parent)
GP = grandparent (and sibs of grandparent)
GGP = greatgrandparent

Of the 1 079 families known to the Centre during 14–25 years and for which there were three known generations of adults 88 or 8 2 per cent presented DM in three consecutive generations of the 253 families known and checked during 23–25 years and for which there were four known generations of adults 3 or 1 2 per cent presented the disease in four consecutive generations

The authors consider that their data speak directly in favour of a true dominant transmission of DM

For a total of 276 transmissions from parent to child (grandparent to parent etc) the sex relations are as follows

from father to son	50
from father to daughter	41
from mother to son	78
from mother to daughter	107
Total	276

With regard to the sex proportion among the affected parents reference is made to a previous study (Pavel Pieptea & Vasilescu 1966) according to which the predominant role of the mothers in the transmission of DM is ascribable to selective factors of a non genetic nature (cf p 228)

From the five pedigrees reproduced in the paper (one over three generations four over four generations) the following transmission lines can be seen

from father to son	5
from father to daughter	5
from mother to son	8
from mother to daughter	15
Total	33

On the basis of his findings that diabetics have more synalbumin antagonism to insulin than have normal subjects Vallance Owen (1966) studied the synalbumin antagonism among relatives of diabetics

Ninety seven members of nine families were examined of whom 58 were found to to be synalbumin positive (of these 16 had overt carbohydrate *intolerance) In addition* the author studied 206 other persons viz healthy volunteers patients with ischaemic heart disease and unselected hospital patients suffering from a variety of other conditions The author gives a diagram concerning the glucose uptake values (above the basal level) for these 303 individuals the diagram shows a bimodal distribution from which fact it is concluded that two phenotypes exist in the population synalbumin positive and synalbumin negative individuals This in turn suggests inheritance of two alternative alleles at a single locus

Vallance-Owen s paper contains pedigrees for the nine case families On the following grounds the author concludes that excessive synalbumin antagonism (the state of being synalbumin positive) is inherited as an autosomal dominant character

(a) There are several single pedigrees covering three generations including both synalbumin positive and synalbumin negative members which have the typical characteristics of this type of inheritance

(b) Synalbumin positive individuals have always had a similarly affected parent so far as it has been possible to ascertain this *in these family series*

(c) There is a 1 1 distribution of affected members in the sibships available for analysis

According to Vallance Owen the observations indicate that there are many constituted diabetics who will never develop carbohydrate intolerance and that there are many more people so constituted than had been previously realized Whether or not a synalbumin positive individual develops carbohydrate intolerance may well depend upon environmental and physiological factors for the synalbumin antagonist is dependent upon the pituitary adrenal system The already increased antagonism can be even further increased under certain conditions notably the growth spurt pregnancy infection the menopause mental stress or when adrenal corticosteroids are administered

The results obtained by Vallance Owen are highly interesting (and highly stimulating for further research in the genetics of DM) However there are some questions to be asked in respect of the data

Of 98 hospital patients selected at random 25 were synalbumin positive However the bimodal distribution of the glucose uptake values seems to indicate that the prevalence in the general population of synalbumin positive individuals is much higher than 25/98 or 27 per cent The distribution covers 97 individuals from the case families of whom 58 were synalbumin positive and a further 206 individuals So far as

can be seen from the diagram more than half of these 206 individuals must have been synalbumin positive

Further the composition of the matings in the nine case families appears to be very peculiar There are in all 21 matings in which *one* of the spouses is synalbumin positive (6 males 15 females) in 15 the other spouse is synalbumin negative (9 males 6 females), and in the remaining 6 the spouse was not tested (6 males) Among the parents of synalbumin positive children in 18 matings *one* parent is synalbumin positive in 13 the other parent is synalbumin negative and in 5 was not tested Such a distribution must be regarded as extremely unlikely if the prevalence of synalbumin-positive individuals in the general population exceeds 50 per cent and it is also very unlikely if the prevalence is of the order of magnitude 27 per cent

It may be remarked that a definite proof of Vallance Owen s theory of an autosomal dominant gene (synalbumin-positive) as being necessary for the development of essential DM does not *per se* solve the problems connected with the genetics of DM Irrespective of whether the prevalence of persons carrying this autosomal dominant gene exceeds 50 per cent or is lower (in the order of magnitude 27 per cent) there remain the circumstances that the morbidity risk for clinically manifest DM is about 6 5 per cent for males and about 13 per cent for females Hence one will have to resort to supplementary hypotheses to explain the gap between the prevalence of the synalbumin positive genotype and the morbidity risk for DM among females and to explain the further deficit among males *One* possible hypothesis would be that there are different genes—a "main gene' for developing DM and a "conditioning gene for being synalbumin positive (or, which is the same an inhibitory gene for being synalbumin negative) A consequence of this hypothesis would then be that, if the prevalence of the synalbumin positive genotype is 50 per cent the prevalence of the main DM genotype among females would be not 13 per cent but 26 per cent In a way this higher prevalence would give support to the theory that the "main' DM gene might be sex linked, since it will be the more easy to explain the occurrence of affected pairs father son the more frequent the DM gene is in the general population However speculations of this kind should preferably be postponed until there are sufficient data concerning the frequency in the general population of synalbumin-positive individuals [1]

Renold and Cahill Jr (1966) give a concentrated account of genetic considerations

[1] Arvill Westberg, Jonsson Hood and Ahrén (1966) report the testing of human albumin prepared by means of two different procedures from blood of seven normal persons and three diabetic subjects viz. the glucose uptake of the rat diaphragm and the distribution of α amino-iso-butyric acid in the intact levator ani muscle of immature male rats Albumin fractions prepared in accordance with the trichloroacetic acid ethanol procedure used by Vallance-Owen showed in some cases an insulin antagonistic activity whereas other fractions did not show any insulin antagonism Albumin prepared as described by Michael (1962) constantly showed a high insulin like activity but no antagonism Albumin preparations from diabetic subjects examined in accordance with Vallance-Owen did not show insulin antagonism

in respect of DM (with a good bibliography) They discuss the well known diabetes surveys by *Wilkerson and Krall (1947 1953) and* Wilkerson Krall and Butler (1959) from Oxford Mass and review evidence for the hereditary nature of diabetic potential — twin studies by Then Bergh (1938 1939) and White (1959) the genealogical studies by Hanhart (1950 1951 1953) and the investigations by Penrose and Watson (1945) Harris (1950) Steinberg (1955 1959 1961) *and Steinberg and Wilder (1952) The* authors summarize the genetic aspects as follows

1 Diabetes mellitus may be defined as a metabolic disorder characterized by hyper glycemia Although hyperglycemia is not present from birth in the great majority of instances there is excellent evidence to in dicate that the potentiality to develop dia betes mellitus is inherited Best available ev idence suggests that the mode of inheritance is a simple recessive one with incomplete penetrance As yet this evidence is not con clusive

2 Genealogies which do not seem con sistent with the simple recessive mode of inheritance may be *the result of the very* high incidence of the diabetic gene com bined with inability to recognize its pres ence prior to the development of hyper glycemia It has been estimated that in the United States about 5 per cent of the popula tion is homozygous (dd) for the gene deter mining the susceptibility for diabetes Avail able information does not suggest that the *major* clinical forms of diabetes are geneti cally distinct the rare syndrome of lipo atrophic diabetes being a probable exception

In a very interesting paper on multifactorial inheritance in relation to nor mal and abnormal traits Fraser Roberts (1961) discusses the genetic component in the causation of common diseases (primarily against the background of data on arterial pressures essential hypertension and duodenal ulceration) He states Theoretically it might be maintained that a gene producing its effect after reproductive life might be come common, for natural selection is not operating against it It might even be argued perhaps, that those whose forbears do not live too long are at selective advantage But in fact the diseases and mortality of post reproductive life show little if any evidence of simple Mendelian ratios Fraser Roberts quotes the findings of Doll and Buch (1950) that 11 5 per cent of brothers of men with duodenal ulceration were similarly affected against an expected figure of 5 5 per cent in the general population of comparable ages and then continues "In instances such as these it is possible to invoke a dominant or intermediate, gene of low penetrance It is true that the presence or absence of disease is an all or none phenomenon but there could be a *threshold and it seems more* plausible to suppose that as a general rule degrees of underlying genetic resistance or susceptibility will ultimately be found to be multifactorial"

However although these statements undoubtedly have a wide bearing there *is now* evidence that *mono*hybrid Mendelian inheritance of diseases with a high age at onset occurs a well known *example is Huntington's chorea* The same applies to *essential tremor* and *essential torsion dystonia* (autosomal dominance Larsson & Sjögren 1960 1966) and to *essential senile dementia* (probably autosomal dominance Larsson Sjogren & Jacobson 1963)

Widukind Lenz (1961) emphasizes the fact that in general the action of a homo

zygotic gene pair is extremely constant, there are practically no examples of recessive diseases with irregular penetrance or greatly variable expressivity

It might be argued that against the background scheduled by Lenz, the general clinical picture of DM—the greatly different course in respect of the occurrence of late complications and, in particular the variability with regard to the age at onset—deviates so much from the ordinary picture found in diseases that are conditioned by monohybrid recessive genes that this mode of inheritance should not be considered at all However with the data hitherto reported in the literature the hypothesis of autosomal dominance will be as good as or better than the hypothesis of autosomal recessiveness [1] Hence irrespective of the significance of Lenz's arguments—which in fact seems to be great—they cannot be used against the theory that DM is conditioned by a major gene but only to rule out the theory that this gene is recessive

A common problem in human genetics—and in medical statistics in general—is the ascertainment of phenotypes In a sense Fraser Roberts's statement that the presence or absence of disease is an all or none phenomenon may be regarded as being connected with the definition of disease for statistical purposes more than with the biological aspects at least in the case of diseases with insidious onset the threshold concept is important—or rather is necessary Often at a certain point of time it is impossible to make sure whether a certain person is afflicted with a certain disease or not at a later period of time it may be possible not only to diagnose the disease with a high degree of accuracy but also to ascertain that it began several years earlier In practice, this situation occurs to a large extent in respect of DM with onset at higher ages Further, even in respect of clinical DM the criteria used for the delimitation may vary, where "prospective" cases ('prediabetes') are included the definitions may become vague or even inappropriate for statistical analysis

Sex differences

It has been shown that the morbidity risks for DM must be much higher than has been presumed in earlier analyses of the genetics of DM, and that for females the total morbidity risk (or the aggregate risk up to age 80 or 90) is about twice the corresponding risk for males Against this background it is very tempting to proceed to speculate whether the higher morbidity risk for females is in some way *sex-connected* (or to a certain extent sex limited)—for instance through effects of pregnancy, estrogenic hormones or the like—or whether the explanation might be that DM is (at

[1] In respect of common diseases with onset at advanced ages a differentiation between dominant and recessive inheritance is very difficult to perform by means of comparisons between the incidence figures registered among parents sibs and children of affected individuals (cf Larsson Sjögren & Jacobson 1963 Larsson & Sjögren 1966) This fact has often been overlooked in the discussions concerning the possible genetic causation of DM (viz. whether it might be ascribable to dominance or recessiveness of an autosomal major gene) —Numerical data are given in Table 82 p 231

least in the majority of cases) conditioned by a *sex linked* dominant gene (located on the X chromosome)

Among factors which are known or may be suspected to influence the onset or course of DM (or both) mention may in the present connection be made of the following

(a) pregnancy, delivery and gestation menstruation and menopause which are strictly limited to the female sex

(b) puberty, which in a sense is more sudden in the female than in the male sex

(c) endocrine disturbances thyrotoxicosis etc, some of which are more frequent among females than among males

(d) muscular work, which is considered to be on average heavier among males than among females (although at higher ages the opposite situation may apply)

(e) overweight which is considered to be more frequent among elderly women than among elderly men (although at younger adult ages the opposite situation may apply),

(f) diseases of the circulatory system which are more frequent or at any rate more dangerous among males than among females

With unbiased statistical series—or series for which the biases can be evaluated—relating to the incidence and prevalence of DM by sex and age, marital status and number of children (or pregnancies) for women and the type of occupation it would be possible to investigate the theory of a sex connection of DM in these respects The problems concerning the significance of overweight and concurring diseases for sex differences in the incidence of DM are more complicated not least because the registration (diagnosing) of DM is often made in connection with the occurrence of an intercurrent disease which calls for a visit to a doctor or to a hospital possibly the best way of studying these problems would be to analyse death records[1]

Further, it should be remembered that—according to the results presented in Chapter IV—the incidence of DM must be fairly equal for males and females up to the age of 50 the largest sex differences in incidence (and the highest morbidity risks) are found for the age groups 55–69 (cf Table 32 p 111, and Table 34 p 117)[2]

Malins FitzGerald and Wall (1965) studied the sex ratio (M/F) among newly diagnosed diabetics at the Diabetic Clinic of Birmingham General Hospital over the period 1930–63 They found a marked increase in this ratio from 1945–49 to 1961–63 viz from 0 48 to 0 89 For the three age groups 35–49 50–64 and 65–79 the (standardized) ratios increased from 0 65 to 1 78 from 0 44 to 0 96 and from 0 38 to 0 78 respectively The authors discuss several possible explanations it is stated that the changed pattern does not appear to be the result of any difference in the type of diabetic patients admitted to the hospital

[1] However overweight is recorded in death certificates to only a very small extent and previous diseases are not recorded unless they are reckoned as the primary or a contributory cause of death

[2] It might be remarked that in the older literature it is often stated that DM is more common among men than among women (cf note p 214) It is thus only natural that attention was not given to the possibility that the mode of inheritance for DM might be sex linked *dominance*

Obviously, reliable incidence data may in many connections be more informative than prevalence data not least because they reflect mainly what has happened during a given period Nevertheless it must always be kept in mind that—in respect of a disease like DM—there will often be considerable acceleration and delay effects (cf p 166) Our results with regard to the morbidity risks (which are based on a study of death certificates for the whole of Sweden in 1961–63 and hence relate to the *prevalence* of DM cf Table 34, p 117) show a much lower (standardized) sex ratio M/F than would follow from the Birmingham incidence figures over the period 1930–63

Pavel Pieptea and Vasilescu (1966) studying the transmission of DM in 3,883 hereditary cases, conclude that the disease is more often inherited through the mother (62 per cent) than through the father (38 per cent)[1] The authors are of opinion that there exist diabetogenic extrahereditary influences due to the mother which must be considered in investigation into the genetics of DM

The theory of sex-linked dominance

It is well established that sex-connected factors – or *factors in respect of which there are sex differences in prevalence*—may play a considerable part with regard to the symptomatology of DM the course of the disease the occurrence of complications etc Yet it seems difficult to accept the idea that such factors are the basic constituents for the occurrence of DM since a great many investigations have shown that—at least to a great extent—DM is genetically conditioned

However the problem of the interaction of heredity and sex connected features like those mentioned above is a complicated one Supposing that DM is really conditioned by a major gene and that this is autosomal (dominant or recessive), we have to presume a gene frequency that explains the *highest* prevalence We shall have to seek for sex connected factors which can explain the deficit in the prevalence of DM among males (*inhibiting factors*), not for sex connected factors which may accelerate the onset or augment the prevalence among females (*precipitating factors*) since in the latter case we have to make the additional assumption that to a great extent DM is not conditioned by an aberrant major gene but—at least in females—must often have a purely non genetic origin or be due to multifactorial inheritance Looking at the list above, it seems very difficult to accept that the occurrence of diseases of the circulatory system will counteract the development of DM It is conceivable that hard work might *postpone* the development of DM, and that pregnancy, endocrine disturbances, etc might *accelerate* it, but in that case it seems likely that the sex difference in respect of DM would largely refer to the shape of the risk curves and not to the aggregate risks up to ages 80 or over

Undoubtedly, a *simpler* theory will be that not only the occurrence of DM but also the occurrence of a sex difference in the total morbidity risk for DM is

[1] Cf also the subsequent study by Pavel and Pieptea 1966 (see p 222)

genetically conditioned So far as we know, all earlier analyses of the genetic aspects of DM are based on assumptions of a comparatively low total morbidity risk for the disease (or in other words a *comparatively low prevalence of the aberrant* gene or genes) Further, it has not always been realized or if realized has not been sufficiently taken into account that individuals with the genetic constitution for being able to develop DM are often registered as non-diabetics (because available information has been inadequate or because the probability of developing DM has been underestimated) [1]

Penrose and Watson (1945) investigated a series of 442 diabetic patients each of whom had one or more close relatives who were similarly affected

The analysis of the sibs of these patients revealed a significant tendency in sibships either for the brothers to be diabetic and the sisters to be non-diabetic or for the sisters to be diabetic and the brothers to be non-diabetic The numbers of diabetic pairs were brothers 104 brother and sister 198 sisters 179

According to Penrose and Watson there are at least three plausible genetic explanations for the *tendency of DM to affect* females in one sibship and males in another viz. (1) partial sex linkage of the main genetic factor (2) complete sex linkage of a gene responsible for the diabetic predisposition, comparatively rare but common enough *to alter* sib-pair frequencies significantly and (3) sex linkage of a secondary gene which modifies the severity of the condition as expressed by the age of onset

These three hypotheses are distinguishable by examining the corresponding parent *child* relationships Partial sex linkage should not disturb the frequencies of the parent child pairs A sex linked main factor would alter the frequencies of the types of pairs so that inheritance from father to son would not occur Evidence for a sex linked modifier would be that the age of onset or the severity of the disease is more similar in unlike sexed than in like sexed diabetic parent-child pairs

The authors state that a survey of the literature with reference to the question of the sexes of parent-child affected pairs proved to be disappointing many papers failed to record the sexes of both members of the affected pairs Their own series gives 45 father and son 51 father and daughter 57 mother and son and 78 mother and daughter For seven pedigrees reproduced in the paper, the corresponding numbers are 2 11 8 and 1 respectively

One of these pedigrees recorded by Bortz (1934) contains 10 diabetics An affected man had four affected daughters and two unaffected sons these four daughters had children viz. (a) an affected son and two unaffected daughters (b) an unaffected *daughter (c) an unaffected son and (d) two* affected sons of whom one had an unaffected daughter and four unaffected sons An unaffected sis er and an unaffected brother of the first mentioned man had an affected daughter and an affected son, respectively

All pedigrees show the same general tendency for the unlike-sexed transmitting parent to be affected and the like sexed transmitting parent to be recorded as unaffected "It ■ reasonable to assume that a number of the apparently normal parents in these and other family groups might have proved to be potential diabetics if they had been more thoroughly investigated or if they

[1] It is obvious, that the recording of DM among family members of DM patients is often very *incomplete An example given by Neel and* Schull (1954) may be quoted as an illus ration. A truthful interested and intelligent female DM patient supplied the information that her mother had DM but that her father and her ten sibs had not DM Yet glucose tolerance tests administered to eight of the sibs revealed that four of them had DM —Klim., Meinert Ho and Briese (1967) found that male s udents report lower proportions of families with diabetics among relatives than do female s udents.

had survived a sufficient length of time for the disease to have become manifest

Penrose and Watson conclude that the analysis of the parental relationships in *their series suggests that the hypothesis of a* sex linked modifying gene is the most likely of the three put forward by them. In the majority of the pedigrees, the primary factor might be a single dominant gene. Possibly there is also a summation effect of additive dominant factors.

In our opinion the pedigrees are rather to be interpreted as speaking in favour of monohybrid sex linked dominant inheritance.

Bartels (1953) gives a series of pedigrees one of which deserves to be quoted. An unaffected man whose father and *two sisters presented DM* had an affected son and an unaffected daughter. However his wife had DM. Since, according to our results, the prevalence of the DM genotype among females would be about 13 per cent it must be fairly common that the son of a dia*betic father marries a woman who may* transmit the DM gene to her children.

Pfandler (1964) gives a thorough description of the family studies made by Hanhart and reproduces eleven selected pedigrees from various papers of his. There is no doubt that these pedigrees must be *interpreted as indicating monohybrid autosomal recessive inheritance if DM is* a comparatively rare disease. However a scrutiny of the pedigrees under the assumption that the total morbidity risk for DM is high (about 6.5 per cent among males and 13 per cent among females) gives a *very strong impression* that in these pedigrees the mode of inheritance is monohybrid sex linked dominant.

Probability evaluations

The *simplest* hypothesis will be that all instances of DM can be ascribed to the action of *a unitary single-gene mutation*.[1] Let us investigate the consequences of such a hypothesis in respect of (a) autosomal dominance, (b) autosomal recessiveness and (c) sex linked dominance.

As before let d denote the frequency of a certain dominant gene D and $r = 1 - d$ the frequency of the allelic recessive gene R. With panmixia in the population and the same fertility in all types of mating the following formulae are valid for autosomal dominance and autosomal recessiveness (cf. Larsson & Sjogren 1966).

Autosomal dominance

In the general population the probability G of a certain person being DD or DR (i.e. of the DM genotype) is

$$G = d\,(2-d)$$

For a DD or DR individual, the probabilities of a (certain) child, a (certain) parent or a (certain) sib being DD or DR are

$$C = P = \frac{1+d-d^2}{2-d}$$

$$S = \frac{4+8d-6d^2+d^3}{4(2-d)}$$

[1] In medical genetics there are many examples showing that the same phenotypical picture or very similar pictures may be caused by different single-gene mutations (as for instance in the case with retinitis pigmentosa) and that the same phenotypical picture may be caused by single-gene mutations as well as by exogenous factors (as for instance in the case with microphthalmia and anophthalmia cf. Sjögren & Larsson 1949).

For different values of G (the frequency of the DM genotype) in the interval 0.01 to 0.25 the corresponding values of d P and S are shown in Table 82

Table 82 Probability evaluations for autosomal dominance and autosomal recessiveness (from Larsson & Sjögren 1966)

G G	Autosomal dominance			Autosomal recessiveness	
	d	P	S	r P	S
0.01	0.0050	0.5038	0.5044	0.1000	0.3025
0.02	0.0101	0.5075	0.5088	0.1414	0.3257
0.03	0.0151	0.5113	0.5132	0.1732	0.3441
0.04	0.0202	0.5151	0.5176	0.2000	0.3600
0.05	0.0253	0.5189	0.5220	0.2236	0.3743
0.06	0.0305	0.5227	0.5264	0.2449	0.3874
0.07	0.0356	0.5266	0.5308	0.2646	0.3998
0.08	0.0408	0.5304	0.5352	0.2828	0.4114
0.09	0.0461	0.5343	0.5396	0.3000	0.4225
0.10	0.0513	0.5381	0.5441	0.3162	0.4331
0.11	0.0566	0.5420	0.5485	0.3317	0.4434
0.12	0.0619	0.5459	0.5530	0.3464	0.4532
0.13	0.0673	0.5499	0.5574	0.3606	0.4628
0.14	0.0726	0.5538	0.5619	0.3742	0.4721
0.15	0.0780	0.5577	0.5664	0.3873	0.4812
0.16	0.0835	0.5617	0.5709	0.4000	0.4900
0.17	0.0890	0.5657	0.5753	0.4123	0.4986
0.18	0.0945	0.5697	0.5798	0.4243	0.5072
0.19	0.1000	0.5737	0.5843	0.4359	0.5155
0.20	0.1056	0.5777	0.5889	0.4472	0.5236
0.21	0.1112	0.5817	0.5934	0.4583	0.5317
0.22	0.1168	0.5858	0.5979	0.4690	0.5395
0.23	0.1225	0.5899	0.6024	0.4796	0.5473
0.24	0.1282	0.5940	0.6070	0.4899	0.5550
0.25	0.1340	0.5981	0.6115	0.5000	0.5625

G G Probability for the general population of an individual being DD or DR (G) or being RR (G′)

Autosomal dominance

d Frequency of the dominant gene D in the general population
P Probability of a parent of a DD or DR individual being DD or DR
S Probability of a sib of a DD or DR individual being DD or DR

Autosomal recessiveness

r Frequency of the recessive gene R in the general population
P Probability of a parent of an RR individual being RR
S Probability of a sib of an RR individual being RR

Autosomal recessiveness

In the general population, the probability G of a certain person being RR (i e of the DM genotype) is

$$G = r$$

For an RR individual the probabilities of a (certain) child a (certain) parent or a (certain) sib being RR are respectively

$$C = P = r$$

$$S = \frac{(1+r)^2}{4}$$

Mating (Father × Mother)	Frequency of mating	Probability of a child being D, DD or DR		Frequency of children being D, DD or DR	
		Son	Daughter	Sons	Daughters
D × DD	d^3	1	1	d^3	d^3
D × DR	$2d^2r$	1/2	1	d^2r	$2d^2r$
D × RR	dr^2	–	1	–	dr^2
R × DD	d^2r	1	1	d^2r	d^2r
R × DR	$2dr^2$	1/2	1/2	dr^2	dr^2
R × RR	r^3	–	–	–	–
Total	1			d	$d(1+r)$

For different values of G (the frequency of the DM genotype) in the interval 0.01 to 0.25 the corresponding values of r, P′ and S are shown in Table 82.

Sex-linked dominance

With the notations previously adopted and assuming panmixia in the population and the same fertility in all types of mating, we get the simple scheme for sex linked dominance given at the top of this page.

With the aid of this scheme we can easily calculate the following probabilities.

In the general population, the probabilities G_m and G_f of a certain male being D, and a certain female being DD or DR are

$$G_m = d$$
$$G_f' = d(2-d)$$

For a D male and for a DD or DR female the table below shows the probabilities of a (certain) son, the father and a (certain) brother being D, and a (certain) daughter, the mother and a (certain) sister being DD or DR—the index before the capital letter denoting the sex of the proband, and the index after it denoting the sex of the relative in question. At the bottom of the table there are given the averages for relatives with the simplified assumption that among the children and sibs of probands both sexes are equally frequent.

Relative	Male proband	Female proband
son or father	${}_mC_m = {}_mP_m = d$	${}_fC_m = {}_fP_m = \frac{1}{2-d}$
daughter or mother	${}_mC_f = {}_mP_f = 1$	${}_fC_f = {}_fP_f = \frac{1+d-d^2}{2-d}$
brother	${}_mS_m = \frac{1+d}{2}$	${}_fS_m = \frac{1+2d-d^2}{2(2-d)}$
sister	${}_mS_f = \frac{1+2d-d^2}{2}$	${}_fS_f = \frac{3-d^2}{2(2-d)}$
child or parent	${}_mC = {}_mP = \frac{1+d}{2}$	${}_fC = {}_fP = \frac{1+d}{2}$
sib	${}_mS = \frac{2+3d-d^2}{4}$	${}_fS = \frac{1+d}{2}$

C, P, S Probability for a child, parent or sib of a proband being D (male) or DD or DR (female)

In the general population the probabilities of a male being D and of a female being DD or DR are d and $d(2-d)$ respectively, and hence—if the sex proportion in the population is $g/(1-g)$—the sex proportion among the carriers of the D gene will be $g/(1-g)(2-d)$. Neglecting the deviation from equality in the general population and hence putting $g/(1-g) = 1$ we get for a random sample of probands

Relative	Male and female probands taken together (with frequency of males $\frac{1}{3-d}$ females $\frac{2-d}{3-d}$)
son or father	$C_m = P_m = \frac{1+d}{3-d}$
daughter or mother	$C_f = P_f = \frac{2+d-d^2}{3-d}$
brother	$S_m = \frac{2+3d-d^2}{2(3-d)}$
sister	$S_f = \frac{2+d-d^2}{3-d}$
child or parent	$C = P = \frac{1+d}{2}$
sib	$S = \frac{6+5d-3d^2}{4(3-d)}$

Numerical example

When investigating the theory of a genetic aetiology for DM we can assume —in accordance with the results obtained in Chapter IV

(a) Autosomal dominance $G = 0.13$
(b) Autosomal recessiveness $G = 0.13$
(c) Sex linked dominance $G_f = 0.13$

The assumptions for G and G imply that—for one reason or another—there must be an inhibition of the manifestation of DM among males. Assumptions which are consistent with the findings for males, viz. G and G = 0.065 would imply that we reject the theory that DM is caused by a single gene mutation: about half of all instances of DM among females (but none among males) would then have to be explained as being caused by exogenous factors (or by multifactorial inheritance). However, it is hard to accept either of these explanations. As already emphasized, the morbidity risks are fairly similar for males and females up to about age 50 and it seems rather difficult to find arguments to support a theory of the existence of strong acting inhibitors among males at the higher ages. Since the greater part of the total morbidity risk refers to ages above 55, it seems highly improbable that exogenous factors or multifactorial inheritance could double the risk for females but not be active at all in respect of males. From these aspects the theory of sex linked dominance does not cause any trouble: one does not have to resort to any assumptions concerning different degrees of manifestation in males and females, or concerning different aetiological patterns for the two sexes.

With the G values given above we get the figures shown in Table 83.

As will be seen from Table 83, the discrimination between *autosomal dominance and* autosomal recessiveness may be difficult to perform by means of the study of the frequency of DM among parents, children and sibs of afflicted persons: in parents and children the expectations for the DM genotype are 0.55 and 0.36, respectively, and in sibs they are 0.56 and 0.46.

In the same way it is even more difficult to differentiate between autosomal and

Table 83 Probability evaluations for sex linked dominance with frequency of DM genotype among females 13 per cent

	Frequency				
	Autosomal dom nance M F	Autosomal recess veness M F	Sex l nked dom nance		
			M	F	M + F
General population					
DM genotype	*0 13*	*0 13*	0 0673	*0 13*	0 0986
DM gene	0 0673	0 3606	0 0673	0 0673	0 0673
Relative of person (proband) with DM genotype					
Father son			0 0673	0 5174	0 3639
Mother daughter			1	0 5499	0 7034
Brother			0 5336	0 2924	0 3746
Sister			0 5650	0 7750	0 7034
Parent child	0 5499	0 3606	0 5336	0 5336	0 5336
Sib	0 5574	0 4628	0 5493	0 5336	0 5390

Italics Assumption concerning the frequency of DM genotype in accordance with the results in Chapter IV (cf Table 34 p 117) in the case of autosomal inheritance the *highest* figures (viz. those for females) are applied

In taking (weighted) averages the sex proportion among children is assumed to be equal for the DM genotype in the case of sex linked dominance the proportion M/F is $1/(2-d)$ = 0 3410 0 6590

sex linked inheritance when the observation series are given without division by sex (the largest inequalities being the expecta tions 0 36 for autosomal recessiveness and 0 53 for sex linked dominance in respect of parents and children and the expectations 0 46 for autosomal recessiveness and 0 54 for sex linked dominance in respect of sibs)

Further it can be seen from Table 83 that in family analysis of sex linked dominance the differences between relatives—males and females taken together—of male probands and relatives of female probands are very small (the largest inequality being the expectations 0 55 and 0 53 in respect of sibs) A family analysis of the differences between brothers and sisters of DM patients —irrespective of their sex—would require a fairly large series (the expectations being 0 37 and 0 70) Theoretically an analysis of *fathers or sons on the one hand and mothers or daughters on the other* (with expectations 0 36 and 0 70) might be somewhat better but it is not easy to arrive at reliable series of this kind the recording of the state of health is often incomplete for the parents and for the children the passed morbidity risk is often only a small fraction of the total risk

Even with a division of the series studied by sex of proband and sex of relative the differentiation between autosomal and sex linked inheritance may be complicated since great problems are involved in ascer taining large family series for which the selective biases can be duly evaluated With sex linked dominance the expectations are 0 29 for brothers of female probands and 0 77 for sisters of female probands The sibs of male probands do not in practice give any differentiation at all (the expecta tions being 0 53 for brothers and 0 56 for sisters)

One effective way of disproving the the ory of sex linked dominance is to study *the parents and children of male diabetics* In a random sample all their mothers and daughters but only 7 per cent of their fathers and sons will be of the DM geno type With unbiased complete registration such disparities between different child parent combinations by sex would be easy to discover However the real circum stances are far more complex In many series the knowledge of DM among parents not only is incomplete but is in complete in an irregular way and there may sometimes be a great many selective factors

of different kinds which are not easy to evaluate For a definite conclusion based on statistics concerning fathers and sons of male diabetics it is important that the ascertainment of the probands shall have been unbiased in so far as the occurrence of DM in the father or the son has not contributed to the registration of the proband A conclusion based on statistics concerning mothers and daughters of male diabetics may be more difficult because the registration of DM may be so incomplete among the mothers and the daughters may be so young that the deviation of the registered prevalence from the theoretical one (viz all belonging to the DM genotype) cannot be ascertained as statistically significant

Genealogical statistics

In all the abundant literature on the heredity of DM very few large series have been published with a simultaneous division by sex of proband and sex of relatives (and also stating the exact relationships between the probands and the relatives in question)

To a great extent, published pedigrees give a clear impression of sex linked dominance *nota bene* if the DM genotype has a comparatively high prevalence in the general population (cf pp 229–230) Of course it should not be overlooked that the morbidity risks (at ages above 50) are considerably higher for females than for males irrespective of the aetiology of DM (conditioned by a major gene or several different major genes due to multifactorial inheritance or environmental factors, combinations of these) there will on average be a higher prevalence of DM among female relatives—of DM probands and of unaffected probands—than among male relatives Due account being taken of the passed morbidity risk, transmission from mother to child will be recorded more frequently than transmission from father to child transmission from paternal grandmother will be recorded more frequently than transmission from paternal grandfather etc Thus there may arise a false impression of sex linked dominance

In their series of 1 631 probands Thompson and Watson (1952) found 39 transmissions from father to son 36 from father to daughter 65 from mother to son and 91 from mother to daughter They emphasize that the tendency for DM to appear more frequently in the like sexed than in the unlike sexed parents of diabetic probands is a statistical phenomenon explicable on the basis of the relative numbers of males and females affected and that (according to their data) no genetic correlation obtains between the sex of the proband and the sex of the affected parent —Among the sibs the number of diabetic pairs were brothers 95 brother and sister 217 sisters 175

As already mentioned only a few series of genealogical statistics on DM have been published with sufficiently complete divisions by sex In the subsequent four sections, the studies by Nilsson (1962 1964 dealing with male probands) and by Harris (1950 cf p 216) will be reviewed and certain data from two of the investigation hospitals (Falun, W Vänersborg, P) will be presented

The Kristianstad studies

In 1953–54 Silwer made a thorough inventory of all known diabetics in Kristianstad County (L) For the County Hospital at Kristianstad which in 1943 was the only one with a special department of internal medicine a search was made of the records

Table 84 Registered prevalence of DM in Kristianstad County (L) by sex, domicile and age 1954 (after Silwer 1958)

Sex and attained age	Number of diabetics			Diabetics per thousand population		
	Total	Rural	Urban	Total	Rural	Urban
Males						
0– 4	2	1	1	0 2	0 1	0 4
5– 9	13	11	2	1 2	1 2	0 9
10–19	32	28	4	1 7	1 9	1 2
20–29	45	38	7	2 5	2 7	1 8
30–39	44	37	7	2 3	2 5	1 7
40–49	81	61	20	4 5	4 2	5 8
50–59	102	86	16	7 2	7 3	7 2
60–69	146	117	29	12 8	12 8	13 0
70–79	114	95	19	16 3	16 0	18 6
80–	19	15	4	9 4	8 8	13 1
Total	598	489	109	4 6	4 6	4 4
Females						
0– 4	1	1	–	0 1	0 1	–
5– 9	12	11	1	1 1	1 3	0 5
10–19	27	22	5	1 6	1 6	1 5
20–29	31	23	8	1 8	1 9	1 7
30–39	33	25	8	1 8	1 8	1 9
40–49	52	43	9	3 0	3 2	2 4
50 59	146	113	33	9 6	9 2	11 4
60–69	229	180	49	18 7	18 1	21 1
70–79	169	131	38	22 0	20 8	28 3
80–	28	21	7	11 0	10 1	15 8
Total	728	570	158	5 7	5 7	5 8
Both sexes						
Total	1 326	1 059	267	5 1	5 1	5 1

of the previous ten years the records of other hospitals in the county where departments of internal medicine were established after 1943 were also searched It appeared that most of the general practitioners in this county usually referred all newly discovered cases of glucosuria or suspected diabetes to a hospital for investigation and care Before the introduction of compulsory health insurance in 1955 all diabetics in the county received insulin free of charge on presentation of a certificate issued by a hospital doctor In addition to the inventory of hospital records questionnaires were sent to all doctors in the county not working in hospitals and also to certain doctors outside Kristianstad County who it was thought might possibly be treating diabetics domiciled in the county [1]

On the cross-section day (March 15 1954) there were registered in all 1 326 known instances of DM (5 1 per thousand population) The data by sex domicile and age are shown in Table 84

As already stated this is a thorough study probably constituting the most complete scrutiny made in Sweden by recourse to hospital records and interviews It should be emphasized that in principle it is a period study over ten years not merely a cross section registration In addition the figures are presented in a direct form related to the proper population data

[1] In an introductory remark Silwer criticizes the use of mortality statistics for deducing data on morbidity to avoid any misunderstanding it should be emphasized that what he is attacking is the use of data on the primary cause of death alone and so far as that is concerned his strictures are quite justified (in respect of DM)

Table 85 Comparison of aggregate morbidity risks according to inventory of Kristianstad County (L) 1954 and with assessment based on mortality statistics 1961–63

Attained Age	Aggregate morbidity risk per thousand				Quotient (1) (2) per cent	
	(1) Calculation based on inventory of Kristianstad County 1954		(2) Assessment based on Swedish mortality statistics 1961–63			
	M	F	M	F	M	F
10	1 4	1 4				
20	2 1	1 8				
30	2 4	1 8				
40	3 4	2 5	12	11	28	23
50	5 8	6 0	21	19	28	31
60	10 0	13 4	34	48	29	28
70	14 5	19 4	47	90	31	22
80			58	117		
90			65	130		

(1) Interpolation from the prevalence figures given by Silwer (1958)
(2) Table 34 p 117

If Silwer s data by sex and age are transferred to give the passed morbidity risks the series given in Table 85 is obtained For comparison the assessments from Chapter IV are shown As will be seen from the table the quotients between the figures from the inventory in Kristianstad County referring to 1954 (in fact a period study) and those from our assessments for all Sweden referring to 1961–63 are of the order of magnitude 0 3 Although—according to Chapter IV—there might be a real underrepresentation of DM in the south western part of Sweden it is quite impossible to modify the assumptions so that the quotients will exceed 0 5 The conclusion is that in spite of the thoroughness observed the scrutiny must have largely underestimated the prevalence of DM (in the age groups relevant here) it would seem viewed relatively that this underestimation is the same for both sexes in rural and urban areas and (at ages below 70 or rather below 75) irrespective of age [1]

Silwer s risk figures were utilized by Sven E Nilsson (1962 1964) in the genetic part of a study on the genetic and constitutional aspects of DM Nilsson deals with three series of probands viz

A 119 males with manifest DM aged 17–25 and resident in Kristianstad County (L) or Malmohus County (M)

Br 237 unaffected males with DM reported in the family aged 18–19 and resident in Kristianstad County

Bc 238 controls to Group Br without DM reported in the family

The registers of the departments of internal medicine in all hospitals in Scania were searched for male diabetics born during the period 1934–42 [2] For these birth groups the military registers were searched for notes on diabetes (reason for exemption from service) In all 154 persons were registered in these ways (104 from both sources 15 from the hospitals alone and 35 from the military registers alone these latter were

[1] According to Nilsson *et al* (1967) the recorded prevalence of DM was 37 per cent higher in 1965 than in 1954 (Silwer s data) the increase was about 43 per cent for males, about 36 per cent for females aged 20–39 or 60–79 and 13 per cent for females aged 40–59

[2] Scania the southernmost province of Sweden covers Malmohus County (M) and Kristianstad County (L)

excluded)[1] Nilsson is of opinion that the 119 probands cover about 90 per cent of all male diabetics in this age group resident in Scania at the end of 1959

In all the number of men liable to registration for military service in Kristianstad County (strictly speaking in the 6th military registration district) amounted in 1959 to 2 834 Excluding 155 who were registered elsewhere and a further 20 who were already exempt on medical grounds and did not *present themselves for registration there* remained 2 659 conscripts For 243 of these DM among relatives (sibs parents sibs of parents grandparents and first cousins) was reported on registration For them, as well as the controls and the probands in series A questionnaires were sent to the mothers inquiring *inter alia* about diabetes among relatives number of relatives of different types and the ages of the proband s parents and grandparents Of the mothers of the probands 89 90 and 71 per cent in series A Br and Bc respectively, cooperated The 18 year-old probands (in series Br and Bc) proved to possess but meagre knowledge of various familial details of relevance In contrast the middle aged mothers gave extensive information on illnesses the members of the family had had on weights etc Of the 243 conscripts primarily assigned to Group Br 15 proved to have been erroneously classified as having diabetic relatives of the 243 controls assigned to Group Bc 9 were reported by their mothers to have diabetic relatives After corrections for these errors the numbers were 237 and 238, as stated above Series Br represents rather less than 9 per cent (237 2659) of the total group (B) from which the *samples are taken*

The number of relatives recorded as affected with DM is shown in the following table allowance should be made for lack of cooperation on the part of the mothers

In testing his observations against expectations (with autosomal recessiveness and autosomal dominance) Nilsson uses the technique of introducing total morbidity risks (aggregate risks up to about age 80 for females) and penetrance factors which

Category of relatives	Number of known diabetics in Series		
	A*	Br	Bc
Brother	7	6	
Sister	2	4	
Father	8	26	
Mother	2	17	
Paternal uncle	10	19	–
Paternal aunt	5	15	1
Maternal uncle	5	11	2
Maternal aunt	3	12	1
Paternal grandfather	7	24	2
Paternal grandmother	7	44	2
Maternal grandfather	7	20	2
Maternal grandmother	8	48	1
First cousin	2	38	–

* Relating to information concerning 101 diabetic conscripts

are different in the series A and B The highest assumptions concerning the prevalence of the DM genotype are 6 25 per cent (recessiveness) and 5 per cent (dominance) Obviously, these prevalence figures are too low and therefore a discussion of Nilsson s calculations will fall outside the scope of the present analysis It may suffice here to quote his conclusion

An autosomal recessive mode of inheritance seemed to be the most likely transmission of diabetes mellitus Then the mutant allele would occur at about 30 per cent of the general population of the investigated area Penetrance would occur during lifetime in about 70 per cent of male and 90 per cent female homozygotes If penetrance occurs among heterozygotes it appears to be low

Dominant inheritance seemed to be less likely but could not be excluded The proportion of pathogenic alleles can then be calculated to be of the order of 0 05 with penetrance among about 25 per cent in male and 30 per cent in female gene carriers "

In Nilsson s series A there are reported 8 fathers with manifest DM whereas—accord

[1] The number of 139 conscripts exempted from military service owing to DM corresponds to 3 per thousand of the total number of male conscripts in Scania

Table 86 *DM relatives of DM probands by sex and age of proband (from data published by Harris 1950)*

Proband s sex and age at onset of DM	Number of probands	Category of relatives														
		Fa	FF	FM	FB	FS	Mo	MF	MM	MB	MS	B	S	So	Da	Total
Number of relatives																
All	1 241	1 241					1,241					3 827	1 418			
Living		453					560					2 967	1,290			
Dead		788					681					860	128			
Number of relatives with DM																
All	1,241	46	10	20	30	25	79	15	25	24	46	67	99	5	5	496
Living		9		3	6	5	17	2	4	5	4	43	67	4	5	174
Dead		37	10	17	24	20	62	13	21	19	42	24	32	1		322
Male proband	536	18	3	11	12	13	24	4	8	9	12	32	28	4	2	180
Living		6	–	2	1	4	11		1	1	1	20	17	3	2	69
Dead		12	3	9	11	9	13	4	7	8	11	12	11	1		111
0– 9	[illegible]	–	1	4	2	1	1		5		1	[illegible]	1			22
10–19	85	6	–	1	4	2	4	1		1	1	2	1			23
20–29	91	1	–	2	2	2	2		1	3	3	7	3			26
30–39	117	4	2	3	3	3	9	2	1	1	2	7	10		2	49
40–49	88	4	–	1	1	5	6	1	1	4	2	6	6	3		40
50–59	75	3	–				2				3	4	6			18
60–69	10	–	–	–									1	1		2
70–79	1	–	–													
Female proband	705	28	7	9	18	12	55	11	17	15	34	35	71	1	3	316
Living		3		1	5	1	6	2	3	4	3	23	50	1	3	105
Dead		25	7	8	13	11	49	9	14	11	31	12	21			211
0– 9	62	1	1	2	4	1		1	2	2	2	1	2			19
10–19	100	3	2	4	5	4	2	3	7	5	4	5				44
20–29	[illegible]	6		1	5		7	2	4	4	[illegible]	5	9			49
30–39	106	6	3		2		18	1	1	2	6	9	11		1	60
40–49	129	3	1		1	5	13	2	1		7	11	21		1	66
50–59	172	8	–	2	1	2	14	2	2	7	9	3	23	1		69
60–69	36	1		–			1					1	5		1	9
70–79	2	–		–												–

Fa father Mo mother FF=father s father FM=father s mother FB father s brother FS=father s sister MF=mother s father etc Br=brother Si=sister So=son, Da daughter — FR (MR)=parents and sibs of father (mother)

ing to our results concerning the passed morbidity risk and assuming sex linked dominance—the expected number should be about 2 (average age about 50 0 021 × 119 = 2 5) In this series there are registered only [redacted] mothers with manifest DM against an expected number of 17 (0 019 × 119 0 13 = 17 4 if the average age of the mothers is 50 if it is 45 the expected number would be about 14) However since 11 per cent of the mothers did not answer the questionnaire the low observed figure might be largely due to selection

In series B there are 6 diabetics among the 2 659 probands 6 affected brothers and 4 affected sisters these figures do not deviate from expectation There are 26 affected fathers against 55 expected but only 17 affected mothers against 50 expected (on the basis of sex linked dominance)

occurrence of DM among the parents of the probands and therefore the recording of 18 affected fathers (6 living 12 deceased) of male probands may be taken as an argument against the theory of sex linked dominance (as the sole aetiology of DM) — It might be observed in addition that there are recorded remarkably few affected mothers of male probands (24 11 living 13 deceased) it does not appear unlikely that the knowledge concerning DM among deceased parents has been more complete for the fathers than for the mothers

A survey of the different types of mating is given in Table 87 (based on the aforementioned appendix) As can be seen from the table there are several cases against the theory of sex linked dominance The recorded transmission is in 12 instances from father to male proband in 4 from father to brother of proband and in another 4 from male proband to son Further there is transmission in 25 instances from unaffected father with affected mother or sib to male proband in 3 from paternal grandfather to male proband and in 5 from paternal grandfather to female proband (In all these instances DM was not recorded in other lines)

Special investigation at Falun (W)

Dr Hogeman has kindly put at our disposal a number of pedigrees in all 277 based on interviews with DM patients and their relatives It should be emphasized that the selection of patients is wholly unbiased from the viewpoint of sex combinations However the number of corresponding patients *without* DM relatives is not available and possibly some patients with an affected sib (but without other DM relatives) have not been recorded

A survey of the data is given in Table 88 As can be seen from a comparison with Table 87, the series is of about the same size as that published by Harris (277 and 336 probands with DM among relatives respectively, 496 relatives with DM in each series)

Where DM is not recorded in other lines, the transmission is in 20 instances from father to male proband, in 4 from father to brother of proband and in 2 from male proband to son Further, there is transmission in 18 instances from unaffected father with affected mother or sib to male proband in 2 from paternal grandfather to male proband and in 3 from paternal grandfather to female proband

Follow-up investigation at Vanersborg (P)

For 818 of the 867 patients included in our clinico statistical study, data on the number of sibs had been registered in the case records For 483 of these 818 patients it was quite clear that no close relative (parent, grandparent sib of parent, sib, child or spouse) had been reported as being afflicted with DM

For the remaining 335 patients the case records were searched (up to the beginning of 1967) and all 'new information was collected Parallel to this patients for whom the information was not sufficiently specified were traced and interviewed Rather many patients had died, but in these cases interviews were made (in person, by telephone or by letter) with close relatives (or, exceptionally, with other persons, for instance a daughter in law)

A survey of the different types of mating recorded for the parents of the 818 probands is given in Table 89

Table 88 DM relatives of DM probands by type of mating (data from Falun W.)

DM among relatives	Number of probands	Number of relatives with DM														
		Fa	FF	FM	FB	FS	Mo	MF	MM	MB	MS	B	S	So	Da	Total
Male proband	(133)	23	3	3	11	15	39	8	17	24	15	29	34	2	3	226
Both sides	4	3	1	–	1	1	2		2			1	1			12
Fa+Mo	2	2	1		–	1	2						1			7
Fa+MR	1	1	–		–				1			1				3
FR+Mo			–													
FR+MR	1		–		1				1							2
Paternal side	40	20	2	3	10	14						6	14		1	70
Fa	20	20		1	2	4						2	10		1	40
FR	20		2	2	8	10						4	4			30
Maternal side	70						37	8	15	24	15	10	13			122
Mo	37						37	1	3	8	5	8	11			73
MR	33							7	12	16	10	2	2			49
Neither side												12	6	2	2	22
Sibs children	19											12	6	2	2	22
None																
Female proband	(144)	30	3	6	11	19	53	2	9	8	31	36	51	5	6	270
Both sides	7	5			1	2	5		1		3	2	5	1		25
Fa+Mo	4	4				1	4					2	3	1		15
Fa+MR	1	1									3					4
FR+Mo	1					1	1						2			4
FR+MR	1		–		1				1							2
Paternal side	46	25	3	6	10	17						9	8			78
Fa	25	25		1	3	5						2	7			43
FR	21		3	5	7	12						7	1			35
Maternal side	65						48	2	8	8	28	11	16	2	5	128
Mo	48						48	2	2	5	19	11	13	1	4	105
MR	17								[illegible]	3	[illegible]		3	1	1	23
Neither side												14	22	2	1	39
Sibs children	26											14	22	2	1	39
None																
Total	(277)	53	[illegible]	9	22	34	92	10	26	32	46	65	85	7	9	496

Abbreviations see Table [illegible] p 239

Where DM is not recorded in other lines the transmission is in 24 instances from father to male proband in 25 from father to brother of proband and in 3 from male proband to son Further there is transmission in 17 instances from unaffected father with affected mother or sib to male proband in 3 from paternal grandfather to male proband and in 3 from paternal grandfather to female proband

It should be noticed that in principle the Vanersborg series is [illegible] *proband registration* and that thus each family [illegible]

Table 89 DM relatives of DM probands by type of mating (data from Vänersborg, P)

DM among relatives	Number of probands	Fa	FF	FM	FB	FS	Mo	MF	MM	MB	MS	Br	Si	So	Da	Total
		Number of relatives with DM														
Male proband	386	28	3	2	11	18	38	3	3	18	16	64	59	3	11	277
Both sides	10	4	–	–	4	2	3	–	1	5	3	1	9	–	–	32
Fa+Mo	1	1	–	–	–	–	1	–	–	–	–	–	–	–	–	2
Fa+MR	3	3	–	–	–	–		–	–	1	2	1	4	–	–	11
FR+Mo	2		–	–	2	–	2	–	–	–	–	–	4	–	–	8
FR+MR	4		–	–	2	2		–	1	4	1	–	1	–	–	11
Paternal side	44	24	3	2	7	16						19	9	–	–	80
Fa	24	24	–	1	1	3						15	6	–	–	50
FR	20		3	1	6	13						4	3	–	–	30
Maternal side	57						35	3	2	13	12	[illegible]	15	1	5	104
Mo	35						35	–	–	8	3	14	12	–	2	74
MR	22							3	2	5	9	4	3	1	3	30
Neither side	275											26	26	2	6	60
Sibs children	46											26	26	2	6	60
None	229															
Female proband	432	28	4	5	12	13	29	4	2	17	21	69	72	8	6	290
Both sides	10	4	–	1	3	4	5	1	–	3	4	5	6	1	–	37
Fa+Mo	3	3	–	–	–	–	3	–	–	–	–	–	1	–	–	7
Fa+MR	1	1	–	–	–	–		–	–	–	1	2	3	–	–	7
FR+Mo	2		–	–	2	–	2	–	–	–	–	3	2	–	–	9
FR+MR	4		–	1	1	4		1	–	3	3	–	–	1	–	14
Paternal side	45	24	4	4	9	9						15	16	–	–	81
Fa	24	24	1	–	3	2						10	7	–	–	47
FR	21		3	4	6	7						5	9	–	–	34
Maternal side	49						29	3	2	14	17	24	14	3	2	103
Mo	24						24	–	1	6	[illegible]	10	7	3	1	58
MR	25							3	1	[illegible]	11	14	7	–	1	45
Neither side	328											25	36	4	4	69
Sibs children	52											25	36	4	4	69
None	276															
Total	818	56	7	7	23	31	67	7	5	35	37	133	131	11	17	567

Abbreviations see Table 86 p 239

counted as many times as there are probands in the sibship further if for instance both the father and a son of his are probands there will be counted two transmissions from father to son (viz an affected son of a male proband and an affected father of a male proband) It is apparent, however, that the registration of probands in a family (because of admission to the investigation hospital) does not occur at random Often the questioning of a patient (and his spouse

and relatives) leads to the admission of other affected family members In addition, it is likely that the observance with regard to DM from the side of the patients and their family members (and the knowledge of the symptoms of the disease) may lead to an earlier detection of DM and—often—to admission to the same hospital

The mean number of sibs of the probands is 5 3 (both for male probands and for female probands) Using the formula given on p 141 the average family size is found to be 3 8 (probands and their sibs)

The exact distributions by age of the parents and by sex and age of the sibs are not known since the registration of the sex and age of unaffected parents and sibs is very incomplete Approximately the distributions in question can be estimated on the basis of the age distribution of the probands and general data concerning the age difference between parent and child and the distribution by sex in different age groups Using these approximate distributions and applying the morbidity risk figures for the general population (Tables 32 and 34) and the probability evaluations in Table 85 the following comparison between observed and expected numbers is obtained

Category	Number of relatives with DM and quotient O E (p r cent)								
	Observed number	Autosomal dominance		Autosomal recessiveness		Sex linked dominance		General population	
	O	E		E		E		E	
Male probands									
Fathers	28	140	20	99	28	17	163	17	163
Mothers	38	135	28	88	43	244	16	32	119
Parents	66	275	24	187	35	261	25	49	135
Brothers	64	81	79	76	84	78	82	10	653
Sisters	59	64	92	53	111	65	91	15	393
Sibs	123	145	85	129	95	143	86	25	496
Female probands									
Fathers	28	169	17	119	24	159	18	21	135
Mothers	29	166	17	109	27	166	17	39	74
Parents	57	335	17	228	25	325	17	60	98
Brothers	[illegible]	111	62	91	76	58	119	13	515
Sisters	72	93	77	77	94	151	48	22	333
Sibs	141	204	69	168	84	209	67	35	403
All probands									
Fathers	56	309	[illegible]	218	26	176	32	38	142
Mothers	67	301	22	197	34	410	16	71	94
Parents	123	610	20	415	30	586	21	109	113
Brothers	133	192	69	167	80	136	[illegible]	23	583
Sisters	131	157	[illegible]	130	101	216	61	37	358
Sibs	264	349	76	297	89	352	75	60	441

The expectation figures are approximations (based on estimated distributions by sex and age) Due account has been taken of the point of time at which the information concerning the occurrence of DM among parents and sibs was obtained

Table 90 Families with at least three affected probands and sibs of probands Vä-

Fam No	Proband			Relatives with DM		Affected sibs		Unaffected sibs		Note
	Sex	Born	Ag	Paternal side	Maternal side	Br	Si	Br	Si	
1	m	1896	D69							
	f	1897	D68	Fa	MS	1+1	1+3	[illegible]	2	Mo died at 91 in 1958
2	m	1882	D84							
	f	1885	L82	FB	Mo	1+0	1+2	2	–	
3	f	1901	L66	FB	Mo	2	1+0	2	3	
4	m	1899	D62	Fa	–	1+3	2	3	1	Mo died at 55 in 1918
5	f	1909	L58	Fa						
				FS	–	3	1+1	1	1	Mo died at 75 in 1949
6	f	1888	L79	Fa						
				FF						
				FB	–	–	1+3	6	1	Mo died at 52 in 1918
7	f	1893	L74	Fa	–	3	1+0	2	2	Mo died at 53 in 1914
8	m	1891	L76	Fa	–	1+2	–	1	4	Mo died at 68 in 1924
9	m	1900	L67	Fa	–	1+2	–	1	1	Mo died at 64 in 1934
10	m	1903	L64	Fa	–	1+1	1	1	1	Mo died at 73 in 1953
11	m	1905	D55							
	m	1914	L53	Fa	–	2+1	–	3	2	Mo died at 61 in 1938
12	m	1928	L39							
	f	1934	L33							
	f	1937	L30	Fa	–	1+0	2+0	1	2	Mo living age 69
13	m	1935	L32	Fa						
				FB						
				FS	–	1+1	1	2	1	Mo living age 54
14	f	1883	L84							
	f	1894	L73	FS	–	2	2+1	3	–	Mo died at 74 in 1936
15	m	1898	L69	FS	–	1+0	2	1	–	Mo died at [illegible] in 1958
16	f	1905	D59	FR	–	–	1+2	2	–	Mo died at 59 in 1936
17	f	1890	D73	–	Mo	4	1+2	1	5	
18	m	1895	D63	–	Mo	1+2	3	2	–	Sister s son DM
19	m	1890	L77							
	f	1901	L66							3 So+Da DM (Fam 21)
	m	1904	L63	–	Mo	2+0	1+1	1	1	Da DM
20	f	1894	N60	–	Mo	1	1+2	5	3	
21	m	1926	L41							

In the series there are in all 44 proband families with at least three affected sibs (probands and sibs of probands) An account of these families is given in Table 90 For each proband are shown sex year of birth and age (at death, or at the latest point of time when information was obtained) For each family data are given concerning relatives with DM (on the paternal side on the maternal side) and number and sex of affected and unaffected sibs

In the 44 families there are 56 probands (27 males, 29 females) and 113 other affected sibs (56 males 57 females) or in all 169 affected sibs (83 males, 86 females), the number of unaffected sibs is 167 (89 males, 78 females)

It should be noticed that the majority of the probands are comparatively old, 16 males (of 27) and 20 females (of 29) are aged 60 or over, 16 males and 18 females were born in 1900 or earlier Against this background it may appear

Fam. No	Proband			Relatives with DM		Affected sibs		Unaffected sibs		Note
	Sex	Born	Age	Paternal side	Maternal side	Br	Si	Br	Si	
	m	1931	L36							
	[illegible]	1933	L34							
	f	1940	L27	–	Mo					
					2 MB					
					MS	3+0	1+0	–	–	
22	f	1882	D84	–	MR	2	1+2	2	[illegible]	
23	[illegible]	1889	N60	–	MR	2	1+2	3	2	
24	[illegible]	1893	L74	–	MB	1+2	1	1	1	
25	f	1901	L66	–	MB	3	1+1	1	–	
26	f	1895	N60	–	MR	1	1+1	–	1	
27	f	1908	D59	–	MF	1	1+1	4	2	
28	f	1885	L82	–	–	4	1+3	2	–	
29	f	1878	D88	–	–	1	1+2	2	5	
30	f	1889	D67	–	–	1	1+2	2	1	Brother's daughter DM
31	f	1880	N72	–	–	–	1+2	1	4	
32	f	1883	N56	–	–	–	1+2	1	1	
33	m	1884	N58							
	m	1898	N46	–	–	2+1	–	1	1	
34	f	1886	N69	–	–	–	1+2	1	2	
35	m	1887	N71	–	–	1+2	–	1	4	
36	m	1888	N62	–	–	1+1	1	1	1	
37	m	1888	D78	–	–	1+2	–	1	3	
[illegible]	m	1889	D76	–	–	1+0	2	3	1	
39	m	1894	D62	–		1+1	1	6	5	
40	f	1897	N58	–	–	–	1+2	4	2	
41	m	1909	L58	–	–	1+0	2	3	2	
42	m	1910	L57	–	–	1+1	1	3	3	2 So+Da DM (Fam. 13)
43	f	1918	L49	–	–	2	1+0	–	1	
44	f	1929	L38	–	–	1	1+1	4	4	

In this table sex of proband is denoted by lower-case letter (m=male f=female)
Age is given at death (D) in 1967 (L) or at last observation (N for families not interviewed)
For affected sibs the table shows the number of probands + the number of other affected sibs
Abbreviations see Table 86 p. 239

peculiar that the sex ratio M F (among the probands as well as among their affected sibs) is almost equal (83 86)[1] Obviously, both the admissions to the hospital and the information obtained with regard to the occurrence of DM among the sibs are (or have been) selective in so far as a larger proportion of the diabetic males than of the diabetic females has been recorded

It is reasonable to assume that the same phenomenon—and to an even higher degree—has applied in respect of the recording of DM among the parents the grandparents and the sibs of the parents

As can be seen from Table 9[illegible] transmission from father to son (without DM on the maternal side also) is recorded in nine families (Fams 4 5, 7-13) in these nine families there a[illegible]

[1] In all there are 3[illegible] probands (cf Table 8[illegible]

and 16 male sibs affected with DM In these families the mother's age (at death or in 1967) is remarkably low (53 54 55 61, 64, 68, 69, 73, 75) Apparently, the arguments against the theory of sex linked dominance are not so strong as might be concluded from a study of Table 89 alone[1]

Discussion

Hitherto there seem to have been no studies performed with a view to investigating whether statistical data on DM and its familial occurrence might be compatible with a theory of sex linked dominance There are several reasons for this

(a) Epidemiological research on DM has largely been concerned with its incidence and prevalence by sex and age, the excess mortality from DM the fertility of diabetics and possible racial geographical and social differences in respect of prevalence, mortality and fertility

(b) Evaluations of the results of family studies have largely been based on the hypothesis that DM is a comparatively rare disease most investigators seem to have tested their empirical data against the theory that the prevalence of the DM genotype is of the order of magnitude 3–4 per cent (or even less)

(c) The shape of the morbidity risk curves has not been sufficiently known As stressed for instance by Jorgensen (cf p 221) the 'age corrections' which are necessary for numerical calculations are complicated and in addition, very uncertain

(d) Most clinicians working on DM have encountered a number of instances where a father and his son are both afflicted with DM In conformity with their conceptions concerning the prevalence of DM, they must regularly have ruled out the possibility of sex linked heredity (if they have considered it at all) the common experience of clinicians is in fact quite sufficient for a definite conclusion that DM cannot to any great extent be conditioned by a sex linked gene with a prevalence of the DM genotype in the general population of 3–4 per cent or less

(e) Although it has been realized that DM is more frequent among females than among males (at higher ages), the discussion among clinicians concerning the sex differences has mainly been focused on possible precipitating factors (pregnancy, hormones etc) on the other hand, geneticists who have analysed material of different kinds on DM have often tried to explain deviations from expected values as resulting from reduced manifestation (incomplete penetrance)

As will have appeared from the data adduced in the preceding sections there *have been recorded comparatively many* transmissions from father to son and comparatively few transmissions from mother to son (viewed against expectations based on a theory that DM is conditioned by a monohybrid sex linked dominant gene) It should be taken into account however, that the series studied are in principle *proband selections* and

[1] Data for a similar scrutiny of the series covered by Tables 87 and 88 are not available

that the registration of probands (as well as the detection of secondary cases among their family members) is in general biased in a very complicated way It is apparent that both the admission rates and the detection rates vary considerably, not only by sex and age but also—which gives rise to a serious evaluation problem—by time

Naturally it will be more difficult to eliminate the theory of sex linked dominance if DM may be caused not only by hereditary factors but also by exogenous factors As already mentioned there are several examples of diseases where—with present knowledge—it is not possible to make a differentiation between cases of *genetic* and *non genetic* origin by means of clinical analysis alone

In the same way it will be more difficult to eliminate the theory of sex linked dominance if DM may be caused also by hereditary factors of another kind (an autosomal major gene multi factorial inheritance) As already mentioned there are several examples of diseases where—with present knowledge—it is not possible to make a differentiation between cases of *different* genetic origin by means of clinical analysis alone

In the general discussion of hereditary factors in DM special attention has been given to the occurrence of DM among the offspring of parents who are both known to have been diabetic. With autosomal recessiveness, all children should be of the DM genotype whereas with autosomal dominance this would apply only to a certain fraction of the children (75 per cent if the DM gene is rare) With sex linked dominance all daughters and a certain fraction of the sons (50 per cent if the DM gene is rare) would belong to the DM genotype Obviously if the study of offspring from these matings cannot discriminate between autosomal recessiveness and autosomal dominance it is not very likely that it could exclude sex linked dominance

Viewed relatively at least the problems involved in the genetic analysis of rare recessive diseases (which are often malignant) and of dominant diseases with early onset (which except for new mutations cannot be very malignant) are fairly simple On the other hand in respect of diseases for which the onset often occurs at higher ages and which are not rare (in the general population or in certain areas) there will as a rule be great difficulty in reaching unambiguous conclusions Very often the observations may be interpreted in different ways a hypothesis that fits the data will not necessarily be true (and very likely it is not true if it is a complicated one and has been specially constructed to fit the data)

As already stated DM is so common a disease that it is difficult to distinguish —by means of statistical analysis of the morbidity among relatives—between autosomal dominance and autosomal recessiveness

The total morbidity risk for DM (the prevalence of the DM genotype) seems to be markedly higher among females than among males On the other hand taking into account the effects of selective migration and selective registration it can be demonstrated that no real sex differences of this kind exist in respect of essential senile dementia (Larsson Sjogren & Jacobson 1963) the quantitatively preponderant forms of cerebrovascular disease (Larsson 1967) essential tremor (Larsson & Sjogren 1960) and torsion dystonia (essential dystonia Larsson & Sjogren 1966)

The two last mentioned diseases are rare in the general population but in the special areas of investigation they are not rare[1] It has been ascertained that these diseases are conditioned by single gene mutations and that for both the mode of inheritance is autosomal dominance the exclusion of the possibility of recessiveness is based on a close scrutiny of pedigrees

Essential senile dementia is conditioned by an aberrant single gene mutation the mode of inheritance being autosomal The prevalence of the genotype in the general population is about 12 per cent (hence in the case of dominance the gene frequency would be 6 per cent and in the case of recessiveness it would be about 35 per cent) There do not exist sufficient data to allow a discrimination between the two possibilities it may be rega d d s a matter of opinion whether a conclusi n on this matter could be based on gener l kno vledge concerning the action of monol ybrid genes

As already ment ned in the parallel study of mor al t f om cerebrovascular disease (Larsso 9 t vas found that the morbidity risks f b ovascular disease do not show any a sex differences In the main—apart from the currence of time lag and selec ive diagnosing —there did not seem to exi t geographical differences In v ew of th general characteristics of the Swedish population and the completeness of the records on causes of death it was suggested that the quantitatively preponderant forms of cerebrovascular disease are to a great extent conditioned by genetic factors—possibly an interaction of autosomal major genes (or even *one* major gene) and multifactorial inheritance The trend figures (and general knowledge concerning the action of genes) were considered to argue in favour of a supposition that these major genes are dominant However a definite statistical proof of these theories is very difficult to accomplish Cerebrovascular diseases are common with onset at advanced ages For deceased patients there generally do not exist detailed hospital records (as is the case in respect of a great many deceased patients afflicted with senile dementia or DM) and the diagnostic demarcation from diseases of the circulatory system has not always been made with sufficient accuracy in respect of diseases of the circulatory system there are great differences between the sexes as well as important geographical differences Finally there are undoubtedly certain environmental factors which play a part in the ultimate breakdown the variation of the monthly figures as well as the differences between ' influenza periods and periods free from influenza give evidence that both climatic conditions and virus infections may precipitate (or accelerate) the onset of an apoplectic insult[2]

The theory that cerebrovascular disease is conditioned by an autosomal monohybrid gene would require that in the general population the frequency of the aberrant gene should be about 0 4 in the case of dominance and about 0 8 in the case of recessiveness With such a high frequency of the aberrant gene the probability that a (certain) sib or a (certain) parent of an afflicted individual has the genotype for developing cerebrovascular disease would be close to 0 8 irrespective of whether the mode of inheritance is dominant or recessive[3] Hence the only way of arriving at conclusive results concerning the mode of inheritance would be the analysis of pedigrees and a thorough study of the outcome of different types of mating

With these examples of diseases with comparatively late onset conditioned by *autosomal* monohybrid genes (of which three are ascertained beyond reasonable

[1] In the general population of the greater part of the parish of X sjo (see map p 4) the prevalence of the tremor genotype was nearly 10 per cent

[2] As is well known there are many resemblances between certain virus infections and the action of aberrant major genes (cf Sjogren & Larsson 1949 Larsson 1965)

[3] Dominance sib 0 798 parent 0 775 recessiveness sib 0 81 parent 0 80

doubt the fourth may at the present be considered as more or less hypothetical) it might be questioned whether it is likely that DM may be conditioned by a sex linked gene However, all four examples refer to the brain, in general it may be presumed that sex differences in respect of inherited diseases of the brain are rare Primarily, DM is connected with hormones and metabolism, possibly also with the structure of body cells and the blood circulation to them Therefore it does not seem *a priori* unlikely that the sex differences in respect of the morbidity risks for DM may be genetically conditioned (hence are sex differences in respect of *the prevalence of the DM genotype*), this of course does not mean that the *shape* of the morbidity risk curves may not be influenced—in males as well as in females—by exogenous factors and by the general genetic equipment of the individual (most likely, in that case, factors connected with multifactorial inheritance)

The theory of sex linked dominance in respect of the majority of instances of clinically manifest DM—*essential diabetes mellitus*—does not exclude either the possibility that there exist morbid states recalling DM which are conditioned by other major genes (dominant or recessive) or the possibility that there exist diseases characterized by carbohydrate intolerance which are caused by environmental factors or are due to multifactorial inheritance

The high morbidity risks recorded for females (at ages above 50) can be considered as a challenge for further research With better material it will be possible to ascertain (beyond reasonable doubt)

(a) that to a fairly great extent DM is due to exogenous factors or

(b) that to a great extent—for one reason or another—DM does not develop in elderly males of the DM genotype or

(c) that a great many elderly males are in fact afflicted with DM or

(d) that to a great extent DM is conditioned by a major sex linked dominant gene

Irrespective of whether one or more than one of these possibilities will prove to be the explanation of the epidemiological data, the significance for future "DM policy is obvious This applies not only to diagnosing and treatment but also for instance to prognostic evaluations genetic counselling the attitudes of diabetics in selecting marriage partners or deciding in respect of reproduction etc

Possibly, thorough *clinical* studies of daughters of diabetic fathers and mothers of diabetic sons may yield conclusive data in respect of the validity of the hypothesis that—at least to a fairly great extent—DM is conditioned by a major sex linked dominant gene and contribute to better knowledge about the clinical variability of the disease [1]

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[1] In respect of diseases with late onset and great variability in the symptom picture it is often impossible to make a sure diagnosis on the basis of clinical observations alone If the disease is found to be hereditary a close scrutiny of pedigrees will make it possible to arrive at a definite diagnosis even for cases with abortive symptoms or otherwise deviating from the normal clinical type (cf Larsson & Sjogren 1960 1966)

The high prevalence of the DM genotype

Before the discovery of insulin (or, more precisely before insulin was taken into general therapeutic use) DM was in many cases a highly malignant disease Irrespective of the mode of inheritance there must have been an antiselection against the aberrant gene, and this for at least two reasons viz (1) the excess mortality among juvenile diabetics and diabetics of reproductive age and (2) the often fatal outcome of pregnancies among diabetic women On the other hand Fraser Roberts may be right in his statement (cf p 225) that it might be argued perhaps, that those whose forbears do not live too long are at selective advantage In other words, the excess mortality among diabetics over say, 50 years of age may not only have been without significance for the transmission of the DM gene but have increased the children s possibilities for reproduction [1]

In a very interesting discussion concerning the pathogenesis of DM Neel (1962, 1964) underlines that there is a considerable body of evidence which suggests that the growth and development of the individual predisposed to DM is accelerated According to Neel this raises the possibility that in the early phases of the natural history of DM there is at some stage during the post prandial cycle a greater than normal availability of insulin resulting in what in agricultural circles would be termed a thrifty animal It is argued that what we now must regard as an over production of insulin antagonists or anti insulins with unfortunate consequences was at an earlier stage in man s evolution, an asset in that it was an important energy-conserving mechanism when food intake was irregular and obesity rare

Neel Fajans Conn and Davidson (1965) hold that the high frequency of the genotype predisposing to so serious a disease as DM presents a genetic riddle

As already mentioned (p 215) von Hofsten discusses the implications of mutation pressure and antiselection in respect of DM he suspects that to a greater extent than geneticists generally assume DM is not hereditary or is ascribable to non specific genes [2]

In a sense, the problems with regard to the high prevalence of the DM genotype and its selective advantages and disadvantages will be much simpler, if it is accepted that the prevalence is high, with high morbidity risks at ages over 50 than if it is assumed that a considerable fraction of the total risk relates to younger ages As will have appeared already (cf Table 34, p 117) the passed morbidity-risk at age 50 is for males about 30 per cent and for females about 15 per cent of the total morbidity risk (the prevalence of the DM genotype in the general population 6 5 per cent for males 13 per cent for females) Therefore the effect of excess mortality before age 50 cannot reasonably have caused more than a modest antiselection against the DM genotype possibly of the magnitude 2–4 per cent per generation

To some extent there must have existed an effect in the opposite direction for mar

[1] In a predominantly agrarian country such as Sweden was until recently there was a social and economic need for the peasants to be married whereas most sons and daughters at home were single (cf Larsson & Sjogren 1966)

[2] It should be remarked that in general the magnitude of the mutation rate (from normal to aberrant gene) is considerably overestimated At any rate this applies in respect of common aberrant genes Presumably the mutation rate from normal to DM gene is *very low*

riages in which both spouses were of the DM genotype (which may be supposed to have comprised 4–5 per cent of all marriages with at least one spouse of the DM genotype) In the case of the wife's death the husband often married a younger woman and thus he got a longer 'reproductive period

The argument has been adduced that formerly the conditions of life—often characterized by hard physical work and scarcity of food—have counteracted the development of DM and that this would explain why the DM gene has not been eliminated It is often stated that the prevalence of DM is far lower in the 'poor' countries than in the highly industrialized countries of the western world Quite apart from the validity of the statistics on which these views are based it is not at all unlikely that exercise and certain dietary precautions may offset or reduce the DM symptoms and possibly also postpone the onset of the disease However as far as Sweden is concerned with its demographic developments and homogeneous population, the similarity of the prevalence data in different parts of the country gives a direct indication that the prevalence of the DM genotype must have been (at least) of approximately the same order of magnitude several hundred years ago as it is nowadays[1]

Inferences from the genetic analysis

Summarizing the results so far obtained the following can be stated

1 The discussions concerning the possibility that DM is conditioned by an aberrant major gene have to a great extent been based on assumptions concerning the prevalence of the DM genotype in the general population of 3–4 per cent (or less) among both males and females Observations have not given decisive results in many series the data are compatible with recessiveness as well as with dominance and incomplete manifestation The possibility of dominant sex linked inheritance does not seem to have been taken into consideration

2 The morbidity risks are considerably higher than has hitherto been generally assumed

3 The total morbidity risk of females is about twice that of males

4 Existing data on the occurrence of DM among relatives of DM patients are not sufficient for a discrimination between recessiveness and dominance to be made

5 There is strong evidence against the theory of an aberrant major gene which in homozygotes causes early onset diabetes and in heterozygotes causes late onset diabetes

6 General experience concerning the action of genes argues against the theory that DM is caused by a recessive major gene

7 With autosomal inheritance the explanation of observed sex differences in respect of the prevalence of DM may be either incomplete manifestation in

[1] Assuming a net antiselection of 3 per cent per generation and disregarding the effects of chance fluctuations it can be calculated that in 1570 when the population of Sweden was 0 9 million the prevalence of the DM genotype would have been about 50 per cent higher than it is nowadays

males or the occurrence of additional cases of exogenous aetiology in females

8 It seems probable that the general response to glucose tolerance tests should be regarded as a graded character, possibly connected with multifactorial inheritance

9 Existing *epidemiological* data can be explained by different genetic hypotheses viz

(a) autosomal dominance, with either *reduced manifestation among males or* the occurrence of exogenous cases—to a fairly large extent—among females,

(b) autosomal recessiveness, with either reduced manifestation among males or the occurrence of exogenous cases—to a fairly large extent—among females,

(c) sex linked dominance without any supplementary hypotheses,

(d) multifactorial inheritance with either reduced manifestation among males or the occurrence of exogenous cases—to a fairly large extent—among females

10 Hitherto no studies seem to have been performed with a view to investigating whether statistical data on DM and its familial occurrence might be compatible with a theory of sex linked dominance

11 As far as we can see existing epidemiological data agree very well with this theory

12 *As a rule, published family data* do not give a sufficient basis for eliminating the hypothesis of sex linked dominance since they rarely use simultaneous division by sex of proband and sex of relative

13 Four series of genealogical statistics are analysed Although these series—in one way or another—seem to give arguments *against* a theory that DM is in general conditioned by a sex linked dominant gene it cannot be ruled out that this result is ascribable to selective recording of probands and selective detection of secondary cases in the families

14 General knowledge concerning the action of dominant major genes and concerning the clinical manifestations of DM does not exclude the possibility that to a certain extent DM may have a nongenetic aetiology On the other hand, it is reasonable to conclude that the majority of all instances of DM should be considered as being conditioned by genetic *factors*

15 The high morbidity risks recorded for females constitute a challenge for research The implications for future DM policy are obvious, irrespective of whether *to a great extent*

(a) DM is due to exogenous factors

(b) DM is conditioned by one or several autosomal genes but does not develop in elderly males,

(c) DM is an equally common disease among elderly males as it is among elderly females or

(d) DM is conditioned by a major sex linked dominant gene

16 *Possibly clinical studies of* *daughters of diabetic fathers, and mothers of diabetic sons, may lead to enhanced knowledge of both the genetic characteristics of DM and the clinical variability of the disease*

Summary and conclusions

1 The present study deals with *clinically manifest diabetes mellitus* (DM). The material used consists of hospital records and mortality statistics, no screening or other population studies were performed.

2 A general discussion of clinical aspects on DM and Swedish practices in the treatment of the disease is given.

3 For the study of hospital patients—inpatients as well as outpatients—there were selected for special reasons, four medium sized general hospitals in different parts of Sweden. These are the county hospitals of Vänersborg (P), Vaxjö (G), Falun (W) and Östersund (Z). In all this material covers 3 759 patients registered at the hospitals as afflicted with DM, mainly during the period 1931–56 with a total of about 45,200 admissions or readmissions and a total of about 19,700 observation years.

4 The admission rates by sex and age and for different parts of the areas covered by the four hospitals are scrutinized in order to evaluate possible selective factors. In other respects, too, a fairly large amount of space is devoted to the analysis of selective factors, in particular those occurring in retrospective studies of clinical material.

5 All patients included in our hospital series have presented both glycosuria and hyperglycaemia (cf p 25). In general, a clearly normal outcome of the oral glucose tolerance tests has been considered to exclude the diagnosis DM. In the hospital series there are only a few patients for whom alternative diagnoses have had to be taken into consideration. Among patients seeking medical advice because of disturbances of the carbohydrate metabolism, glycosuria and subjective diabetic symptoms, the frequency of renal glycosuria has been about 2 per cent. Quite generally it can be stated, that the quantitative significance of the problems of differential diagnostics has been comparatively small.

It should be borne in mind that the frequency figures with regard to the occurrence of different symptoms and complications refer to DM patients admitted to general hospitals during a certain period of time, the composition of a hospital series may be considerably changed, for instance, if "detecting procedures" are undertaken on a large

scale, or if the capacity of the hospitals to take care of new patients is greatly increased However, the effects of such changes will concern the prevalence of intercurrent disease more than DM *per se*, since the main bias in the present series seems to be that many persons with clinically manifest DM were admitted to the hospitals *because* they were afflicted with another disease

In the main the patients admitted to the county hospitals can be regarded as fairly representative of persons who show DM to a degree that—where thorough clinical examination is performed—will be diagnosed as an instance of clinically manifest DM

6 In the hospital series the patients were classified by age at onset of DM into three types viz age at onset 0–14 (juvenile onset), 15–39 (early adult onset) and 40 or over (late onset) As a rule the data in the clinico statistical tables are given with simultaneous division by sex, hospital, onset type and duration (5 year groups, reckoned from the year of onset of DM)

7 In the present series, the control has been better among patients with late onset than among patients with juvenile or early adult onset

8 The DM patients did not present overweight to an extent that exceeds what is to be found in the general population (Whether or not there was a higher frequency of overweight among the DM patients *before* the onset of the disease cannot be determined on the basis of the registered data)

9 With regard to constitutional type the data are consistent with current views that among diabetics the asthenic type preponderates at younger ages, whilst the pyknic type is frequently seen in late onset DM

10 The frequency figures (by sex and age) for hypertension agree fairly well with the frequencies existing in the general population The differences found may in part be due to selective factors and it does not seem justifiable to conclude, on the basis of the data on blood pressure, that DM *per se* causes hypertension

11 In respect of juvenile onset DM, menarche often occurs later than it does in the general population The data indicate that on average the menopause tends to occur somewhat earlier among diabetics than in the general population

The data do not support the theory that the fertility of diabetics, before the onset of DM, is higher than the fertility in corresponding groups of the general population This theory, which is not infrequently advanced in the literature, seems in the main to be based on a misinterpretation of the existing statistical data

After the onset of DM the number of children born to female DM patients is far below the corresponding number for females in the general population

12 The insulin treatment applied cannot be regarded as an expression of a scientifically founded system but rather as representing a series of practical con

siderations, concerning which it might be said that they functioned much better than could justifiably have been expected

There were comparatively large divergences between the attitudes of the physicians with regard to the most suitable insulin treatment—or, rather with regard to what compromises could be considered acceptable

Roughly speaking insulin therapy was applied to all patients with juvenile onset DM to about 90 per cent of the patients with early-adult onset DM and to more than 60 per cent of the patients with late-onset DM (Table 49 p 152) For patients with late-onset DM the frequency figures increase markedly with increasing duration Very likely however this is an expression of selective factors (see p 153)

13 Among patients with infections ketosis was registered for 2 per cent of the males and 4 per cent of the females with late onset DM, for 8 and 12 per cent in the early adult onset type and for 20 and 24 per cent respectively in the juvenile onset type For the juvenile diabetics the frequencies are higher—viz 31 and 32 per cent—in the duration group 0–4 years

During the later part of the investigation period hypoglycaemic coma was seen only exceptionally

14 With the present state of knowledge it has not been possible to arrive at unambiguous conclusions concerning the aetiology of the so-called late complications of DM—retinopathy nephropathy and neuropathy It is obvious however that from the statistical point of view there is a correlation between the duration of DM on the one hand and the prevalence and severity of the vascular changes on the other

There have been divergent opinions concerning the proper prophylactic and therapeutic measures to be taken with regard to the late complications A central topic in the discussions has been the role played by the diet Definite proofs that a strictly regulated diet prevents the late complications or makes their course milder do not seem to have been given

Our data concerning the incidence and prevalence of late complications show conspicuous divergences between the hospitals and even more conspicuous divergences between the observations from different periods of time (epochs) In our opinion, the results obtained from the analysis of the data on late complications in the four hospital series are highly illuminating in so far as they prove the necessity of the utmost caution in the use of hospital register data (even very extensive case records) for conclusions concerning geographical differences and changes with time in the incidence and prevalence of late complications of DM concerning connections with the age of the patients and the duration of the disease and concerning the effect of different treatments applied

DM is a disease where much attention must be given to each individual patient and it would not be advisable to perform prospective studies over long periods involving differential treatment of strictly comparable patients Retrospective studies of the occurrence of late complications in patients who have been treated differently—at different

hospitals or at the same hospital during different periods of time—are often very difficult to evaluate, our statistical analysis gives a series of examples with regard to the biases and other selective factors that might occur in respect of the admission of patients and their readmissions, in respect of the diagnostic criteria applied and in respect of the recording of clinical observations

15 The diagnosis of diabetic retinopathy depends on the definitions used, the available technical resources and the knowledge and competence of the investigator The development of diabetic retinopathy is correlated strongly to the duration of DM and weakly to the patient s age Older reports where these factors are not separated are often not comparable owing to bias in the selection of patients Therefore it is only natural that reports on the general frequency of diabetic retinopathy differ widely

In our series, there is a striking increase with time (epoch) in the total frequency of retinopathy The increase appears uniformly for all durations including the first years after onset, and therefore cannot be explained by unfortunate consequences of modern treatment The main effect must be ascribed to general progress in the diagnostics of retinopathy and to more complete registration in the case records

The frequency of retinopathy, as diagnosed at Swedish county hospitals seems to be best represented by the figures given in Table 60 (p 177)

From our data it is not possible to prove or disprove connections between treatment and the development of retinopathy Nevertheless, the data seem to support the theory that no treatment hitherto used in general hospitals will decisively improve or retard the development of retinopathic changes among the diabetics

16 There is an increase with time (epoch) in the total frequency of nephropathy However, as can be seen from Table 71, p 188 the time variation is small compared with the almost startling differences between the investigation hospitals, the (standardized) prevalence found at Vänersborg (P) being about six times the prevalence recorded at Östersund (Z)

17 The prevalence of neuropathy shows the same general pattern as does the prevalence of retinopathy and nephropathy There are great differences between the investigation hospitals (see Table 75, p 194) With the concept adopted at Falun (W) and Östersund (Z), the overall frequency of neuropathy would have been about 26 per cent

The general features of the frequency of neuropathy are not incompatible with a hypothesis that this frequency depends not on the duration of DM but only on the patient's age (cf Table 76 p 194)

18 With regard to vascular lesions other than retinopathy and nephropathy, only slight deviations from pure age dependence were found The data in our series do not give rise to a supposition that these other vascular lesions found in diabetics should *generally* be regarded as sequelae of DM

19 A study of the mortality among the patients in the hospital series during the 10-year period 1946–55 was made covering in all 16 440 observation years and 398 deaths during this period

Compared with the population mortality, the diabetics in the hospital series show a mortality ratio of about 1 4 (all ages taken together) For juvenile diabetics the mortality ratio is considerably higher (order of magnitude 6 0) and for diabetics with age at onset 40 or over it is about 1 3

20 A special processing of all death certificates in Sweden 1961–63 was per formed, covering primary causes of death as well as contributory causes and complications *In all there were registered* 3,324 deaths with DM as the primary cause and 7 273 deaths with DM as a contributory cause

The period 1961–63 presented nor mal" mortality (viewed against the trend) and it cannot reasonably be suspected that the data are disturbed by any acceleration or delay effects

For comparison data on mortality and causes of death based on official statistics, are discussed

In the analysis of the data from the death certificates for the 3-year period 1961–63 the resulting statistics were studied in detail with regard to sex, age *the primary* cause of death and *domicile*

There exist gaps in the death certi ficates connected with variations of the completeness of the registrations by age primary cause of death and domicile The prevalence of DM recorded in the death certificates is lower in Stockholm City and in the south western part of Sweden than in other parts of the country It is lower in the highest age groups than at ages 70–74 and 75–79 The registration of DM as a contributory cause is less frequent for deaths from the primary causes neoplasms and violence than for deaths from other primary causes

21 On the basis of a detailed scrutiny of the data the most adequate estimate is found to be that the total morbidity risk for clinically manifest DM (the aggregate morbidity risk up to age 90) is about 6 5 per cent for males and about 13 per cent for females The aggregate risk up to age 50 is 2 1 per cent for males and 1 9 per cent for females and up to age 70 it is 4 7 per cent for males and 9 0 *per cent for females* The life table expectancy at birth for contracting clini cally manifest DM is calculated at 4 8 per cent for males and 10 3 per cent for females

22 There has been an increase in the number of first admissions with time especially in the higher age groups In part this is a reflection of the changing age composition of the population (and the increase in population number) but in the main it is ascribable to other factors such as better communications, aug mented hospital facilities and a general rise in the consumption standards in *respect of medical care*

23 It is calculated that at the end of 1965 the number of persons in Sweden with clinically manifest DM was about 159 000 (59 000 males and 100,000 females) or 2 05 per cent of the total population (1 52 per cent for males

2 57 per cent for females) For ages 15 and over, the percentage is 2 60 (1 95 for males, 3 24 for females)

As a result of the changing age structure and the growth of population the number of diabetics in Sweden will show an annual increase by about 0 7 per cent for males and 1 4 per cent for females or by about 2,000 a year

24 The overall excess mortality among persons with clinically manifest DM cannot reasonably exceed 15 per cent, this, however, does not preclude that at ages below 50 the average excess mortality can be 200 per cent or more

25 The rating rules applied by the Swedish life and health insurance companies with regard to DM are presented and the problems connected with the insurance of diabetics are discussed

The rating of DM risks in individual insurance has been gradually liberalized especially in revisions undertaken in 1964 and 1966 Suggestions with regard to further liberalization are adduced

The rapid growth of group life and group health insurance has largely contributed to the effect that diabetics nowadays generally have considerable insurance protection as belonging to groups for which the participation is secured through agreements between the parties of the labour market through legislation (for state employees) or through membership of an organization As a rule diabetics obtain group life insurance without any restrictions at all, if they possess working capacity when joining the group

It is probable that in Sweden the average diabetic has a higher life and health insurance coverage and more liberal insurance conditions than is the case in most other countries

26 Current conceptions with regard to the genetic aetiology of DM are reviewed and analysed The discussions of the possibility that DM is conditioned by an autosomal major gene have regularly been based on assumptions concerning the prevalence of the DM genotype in the general population of 3–4 per cent (or less) among both males and females Observations have not given decisive results, in many series the data are compatible with recessiveness as well as with dominance and incomplete manifestation Existing data on the occurrence of DM among relatives of DM probands are not sufficient for a discrimination between recessiveness and dominance to be made

However, as is evident from our analysis of mortality statistics (death certificates), the morbidity risks for DM are far greater than has hitherto been considered to be the case and are far greater among females than among males The implications of these findings with regard to the theories concerning the genetic aetiology of DM are analysed

27 Hitherto no studies seem to have been performed with a view to investigating whether epidemiological and genealogical data on DM and its familial occurrence might be compatible with a theory of *sex linked dominance*

Existing epidemiological data can be explained by different genetic hypotheses

(a) autosomal dominance, with either reduced manifestation among males or the occurrence of exogenous cases among females,

(b) autosomal recessiveness with either reduced manifestation among males or the occurrence of exogenous cases among females

(c) sex linked dominance

(d) multifactorial inheritance with either reduced manifestation among males or the occurrence of exogenous cases among females

According to our results from the analysis of death certificates *epidemiological statistics* argue distinctly in favour of a theory that hereditary DM is in general conditioned by a monohybrid dominant sex linked gene

Four series of *genealogical statistics* are analysed—two series from the literature by Nilsson (1962 1964) and by Harris (1950), a special investigation at Falun (W) and a follow up investigation at *Vänersborg (P) Although these* four series—in one way or another—seem to give arguments against a theory that DM is in general conditioned by a sex linked major gene it cannot be ruled out that this result is ascribable to selective recording of probands and selective detection of secondary cases in the families

28 General experience concerning the action of genes argues against the theory that DM is conditioned by a recessive major gene

General knowledge concerning the action of dominant major genes and concerning the clinical manifestations of DM does not exclude the possibility that to a certain extent DM may have a non genetic aetiology It seems likely however, that the majority of all instances of DM are conditioned by genetic factors

29 The high morbidity risks recorded for females constitute a challenge for research The implications for future DM policy are obvious irrespective of whether *to a great extent*

(a) DM is due to exogenous factors

(b) DM is conditioned by one or several autosomal genes but does not develop in elderly males

(c) DM is an equally common disease among elderly males as it is among elderly females or

(d) DM is conditioned by a major sex-linked dominant gene

Possibly *clinical* studies of daughters of diabetic fathers and mothers of diabetic sons may lead to enhanced knowledge of both the heredity of DM and the clinical variability of the disease

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SUPPLEMENTUM 478

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Purification of the Substrates — Kinetic and Methodological Studies

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These papers will be referred to by their Roman numerals

Introduction

The transformation of fibrinogen into fibrin is caused by the proteolytic enzyme thrombin, which is the result of a complex chain of promotive as well as inhibitory enzyme reactions (Davie and Ratnoff 1964, Macfarlane 1964 a, 1964 b, 1966) The fibrin in turn is degraded by another proteolytic enzyme, plasmin, which also is the result of a chain of enzyme reactions, also promotive as well as inhibitory In the blood, one may assume that there is a complex equilibrium of all these opposing reactions of activation and inhibition, of polymerization and degradation (Stafford 1964) It has also been suggested (Astrup 1956 a, 1956 b, 1962) that there is a continuous formation and lysis of fibrin

To study these reactions in blood is extremely difficult because of the great complexity of the reactions Quantitative determinations of the individual factors involved are also difficult to perform because all the activating and inhibiting factors are present simultaneously

As some of these factors can be purified, parts of the systems and the isolated reactions can be studied and information as to the course of the reactions can be obtained This information can form the basis for further studies of the same reactions in blood The isolated reactions can also be used to construct specific quantitative methods to determine the various factors

The determination of the individual factors in the coagulation system ultimately depends on the activation of prothrombin into thrombin, which usually is determined by means of the clotting of fibrinogen Likewise, the activity of the individual components of the fibrinolytic system can be assessed on the basis of the amount of plasmin generated from plasminogen during the testing procedure The ability of this plasmin to lyse a fibrin clot, the lysis time method, is often used to determine the amount of plasmin However, in this method the fibrin clot is usually formed in the presence of plasmin Thus, the determination of plasmin by means of fibrin clot lysis involves a combination of the coagulation and the fibrinolysis which increases the complexity of the method

Various other methods exist Those commonly used are based on the ability of plasmin to split casein or synthetic esters However, fibrin is often preferred as substrate because it is the physiological substrate for plasmin and thus expresses the fibrinolytic activity of plasmin The esterolytic activity obtained with synthetic esters as substrates does not seem to be identical with the

fibrinolytic activity (Markus and Ambrus 1960 a)

In the last decade, great interest has also been focused on the plasminogen activator, urokinase, a normal constituent of human urine It has been suggested that urokinase excretion in some way is related to the release of the plasminogen activator from the tissue into the blood or produced by the kidney Therefore, the urokinase excretion has been studied in various diseases, especially in thromboembolic disorders (a review has been made by von Kaulla 1963, chapter 8) Attention has also been drawn to the use of urokinase in therapeutically induced fibrinolysis As a result of the growing significance of urokinase it has become of great importance to develop a sensitive and accurate method for quantitative determination of urokinase

When plasmin, the activated form of plasminogen, is determined by means of the lysis time method an expression of the fibrinolytic activity is obtained Therefore, we have adopted the principles of this method for determination of plasminogen and urokinase

The aim of this work is an analysis of the influence of the various factors participating in the fibrinolysis and kinetics of the urokinase activation of plasminogen The practical performance of the methods is based on the results obtained

In this work the methods have been applied to a "purified system' However, the results of the investigations have also been used to determine plasminogen in plasma (Berg, Korsan-Bengtsen, and Ygge 1966)

If lysis of the fibrin is used to measure plasminogen, it is of great importance that the fibrinogen and thrombin are not contaminated with plasminogen This has been emphasized by Astrup (1956 b), Sherry, Fletcher and Alkjaersig (1959), Flute (1964) and MacKav (1964) If the urokinase amount is determined by means of plasminogen activation it is also essential to have a plasminogen which is soluble at neutral pH and at physiological ionic strength and which has a low spontaneous plasmin activity

In compliance with this, simple methods for preparation of fibrinogen, plasminogen-free thrombin and a soluble plasminogen with a low spontaneous activity have been elaborated

Thus, the work is divided into two parts Part One describes the preparation of the substrates while Part Two discusses the kinetic studies and the assays

PART ONE

Purification of the Substrates

I Separation of human fibrinogen and plasminogen by means of gel filtration

A plasminogen-free fibrinogen is a pre-requisite for using fibrin as substrate in quantitative determinations of plasminogen However, most of the available fibrinogen preparations are contaminated with plasminogen (Blomback and Blomback 1956)

To separate fibrinogen from plasminogen has been difficult According to Remmert and Cohen (1949), plasminogen has a tendency to form complexes with other proteins and this phenomenon has been considered to be responsible for the difficulties (Bergstrom and Wallén 1961)

Several methods to separate fibrinogen from plasminogen have been tried The fibrin-contaminating plasminogen has been inactivated by heating the fibrin to 85° C (Lassen 1953) This method, however, cannot be used for fibrinogen In order to achieve the required purity, Markus and Ambrus (1960 a) found it necessary to repeat the precipitation three times according to the method of Laki (1951) which is based on precipitation with phospate buffer and ammonium sulphate A fibrinogen substantially free from plasminogen and plasmin has been obtained by precipitation with diethylether (Keckwick, Mackay, Naunce and Record 1955) Charcoal, Dacro G 60, has been used by Maxwell, Nickel and Lewandowski (1962) to remove plasminogen from fibrinogen A lysis time of 13—24 hours was obtained Nauninga and Guest (1964) used carbon to remove contaminating plasminogen As l-lysine and ε-aminocaproic acid increase the solubility of plasminogen, precipitation with ethanol in the presence of l-lysine or ε-aminocaproic acid removed the contaminating plasminogen (Bergstrom and Wallen 1961, Mosesson 1962) Separation of plasminogen from fibrinogen by means of ion exchangers of cellulose type has been used by Mosesson and Finlaysson (1963) and Godal and Luscher (1960) using gradient chromatography on DEAE-cellulose Complete separation was not obtained, however We have also tried ion exchangers of the cellulose type but without success Gel filtration on Sephadex G-200 has been used by Straughn and Wagner (1966) to characterize fibrinogen No investigation was made to find out whether the

gel filtration separated plasminogen from fibrinogen. Plasma and Cohn's Fraction I were separated by Lewis (1964) on Sephadex G-200 to estimate the molecular weight of the various coagulation factors and of plasminogen. Our findings of a good separation of plasminogen from fibrinogen by means of Sephadex G-200 were confirmed by him.

The great difference in molecular size between fibrinogen and plasminogen was the reason why we tried separation by means of gel filtration. This method is described in Paper I. A fibrinogen with a high degree of purity (Blombäck *et al.* 1956) was used as starting material. The gel filtration was performed at room temperature on a Sephadex G-200 column with a salt-buffer solution of 0.2 M Tris HCl and 0.2 M NaCl (1:5). A better result was obtained at room temperature than at +4° C. This is in accordance with the findings of Godal *et al.* (1960) on DEAE-cellulose. The fibrinogen and the plasminogen contaminant came in two well separated peaks. The fibrinogen was tested with various urokinase concentrations because it was not known whether high urokinase concentrations inhibited plasminogen activation or not as does streptokinase. The first and the last part of the fibrinogen peak seemed to contain traces of fibrinolytic activity which gave a lysis time of about 4 days compared with more than 14 days for the rest of the peak. The contamination of the last part was certainly caused by overlapping of the plasminogen peak. The cause of the contamination of the first part is not definitely known. One possibility is that plasminogen traces formed complex with fibrinogen and these large molecules came in front of the fibrinogen molecules. However, separation of fibrinogen from greater molecules probably did not take place. Laurent and Killander (1964), however, found an available gel volume for fibrinogen. According to the authors, this was within the error of the method. Straughn *et al.* (1966) found the same volume for fibrinogen as for Dextran 2000 which has a molecular weight of about 2 000 000.

The experiment described in paper I was done with a small column. For purification on a larger scale, a column with a diameter of 10 cm and a length of 100 cm was used. On this column, 1 g fibrinogen could be purified each time.

As the assays in which the fibrinogen was used were performed in buffers of physiological ionic strength and with pH 7.4, the buffer used in the preparation was later changed to NaCl and Tris HCl (1:4), pH 7.4 and ionic strength 0.15 M. The same good separation was obtained.

The preparation of plasminogen-free fibrinogen described in Paper I is easy to perform. If highly purified fibrinogen such as that prepared according to Blombäck *et al.* (1956) is used as a starting material, the procedure consists of one gel filtration on Sephadex G-200. If a large column is used, the purification can be done on a large scale. It is not necessary to add l-lysine or ε-amino caproic acid to the buffer.

II Human and bovine plasminogen-free thrombin purified by means of gel filtration and ion exchange chromatography

To obtain a plasminogen-free clot, it is necessary that both the fibrinogen and the thrombin are free from plasminogen It has been very difficult to separate thrombin completely from plasminogen Even highly purified preparations (Korsan-Bengtsen and Ygge 1961, Magnusson 1965 a) contain plasminogen traces It is believed that some fibrinolytic activity can be ascribed to the thrombin molecule itself (Guest and Ware 1950, Brakman, Klug and Astrup 1964) However, the fibrinolytic activity seems to depend on the degree of purity Thus, preparations of high purity have shown fibrinolytic activity only at high concentrations (Guest *et al* 1950)

Several methods have been tried to prepare plasminogen-free thrombin Acid precipitation was used by Hudeman (1940) Markus and Ambrus (1960 b) used stepwise alcohol precipitation and selective inactivation of the plasminogen by means of β-mercaptoethylenamine Hink and MacDonald (1962) used fractional precipitation with ammonium sulphate while De Vreker, Vermylen and Verstrate (1962) used column chromatography Brakman *et al* (1964) used chromatography on Amberlite IRC-50 All these methods gave preparations with traces of fibrinolytic activity

A method to prepare a plasminogen-free thrombin is described in Paper II The purification was started with adsorption of fresh human or bovine plasma to $BaSO_4$ (Bordet and Delange 1912) As prothrombin adsorbs to $BaSO_4$ while only traces of plasminogen are adsorbed, this procedure separated the main part of the plasminogen from the prothrombin This crude prothrombin was eluted from $BaSO_4$ with citrate Human brain thromboplastine and CaCl were used to activate the human crude prothrombin Calf brain thromboplastine was used to activate the bovine prothrombin After dialysing 24 hours against distilled water and high speed centrifugation the crude thrombin was purified by means of gel filtration on Sephadex G-75 Further purification and concentration were performed by means of adsorption to a small column of the cationic exchanger CM-Sephadex A-50 The buffer was 0 1 M Tris-HCl pH 7 4 Stepwise elution was performed The thrombin was eluted with a salt-buffer solution of 0 5 M NaCl and Tris HCl, pH 8, at the proportion 4 1

Testing with the lysis time method showed that the final preparation did not contain plasminogen Activation was done with urokinase

The commercial thrombin preparation Topostasine , which contains a large plasminogen amount was also used as a starting material This thrombin could not be separated completely from plasminogen

Purification of thrombin on Sephadex G-75, G-50, and G-25

Purification of human prothrombin has been done on Sephadex G-200 in

0.15 M $CaCl_2$ (Magnusson 1965 b). Traces of contaminants could be removed by chromatography on DEAE-cellulose. Purification of thrombin has been done by means of Sephadex G-25 (Strassle 1963). Fibrinolytic activity was found after the peak which contained clotting activity.

In our investigations, gel filtration of the crude thrombin prepared from the eluted $BaSO_4$ adsorbed plasma and of the commercial preparation 'Topostasine' was performed on Sephadex G-75, G-50, and G-25. The best separation of thrombin from the main part of the inert protein was obtained with Sephadex G-75.

The final thrombin preparation contained no plasminogen if crude thrombin prepared from the $BaSO_4$ elution was used as a starting material. This crude thrombin, however, contained only traces of plasminogen and the distribution of plasminogen and thrombin in the elution pattern could not be so clearly demonstrated. The commercial thrombin 'Topostasine', which contains a large plasminogen amount, was used to investigate the separation of thrombin from plasminogen. Overlapping was seen between the plasminogen and the thrombin and the final preparation still contained plasminogen. This overlapping was caused by the plasminogen peak which was elongated compared with the thrombin peak. Thus, the gel filtration with Sephadex G-75 could only separate plasminogen from thrombin if the crude thrombin contained small plasminogen amounts. An elongated plasminogen peak is also observed when euglobulin is gel filtrated with Sephadex G-200 (III). The cause of this is unknown. Protein interaction causing adsorption to other proteins or elongated molecules are probable explanations.

With Sephadex G-75 the thrombin was so well separated from the protein with larger molecules than those of thrombin that it was expected that separation with Sephadex G-50 would also be successful. This was verified.

With Sephadex G-25 thrombin was not separated from protein consisting of larger molecules than those of thrombin. However, there was a peak after the thrombin. The peak contained no fibrinolytic activity. Thus, the findings of Strassle (1963) could not be verified. As the best separation from inert protein was obtained with Sephadex G-75, this was used as the standard method.

Plasminogen seems to have a tendency to adsorb to thrombin because it is difficult to separate it completely from thrombin even with CM-Sephadex although purified plasminogen does not absorb to CM-Sephadex at 0.3 M Tris HCl, pH 7.4.

Gel filtration with Sephadex G-75 seems to be well suited to separate plasminogen from thrombin, because Sephadex G-75 has an exclusion limit at a molecular weight of 40,000 and thrombin has a molecular weight which probably is lower than 30,000 (Magnusson 1965 a, Schrier, Bromfield, and Scheraga 1962, Cohley and Scheraga 1961, Laki and Gladner 1964). Plasminogen possibly has a molecular weight of about 100,000.

Separation with gel filtration is de-

pendent on the size of molecules (Laurent and Killander 1964) The separation obtained with Sephadex G-50, which has an exclusion limit at a molecular weight of about 10,000, indicates that the molecular weight of thrombin is lower than 10,000 As no separation from greater molecules than those of thrombin was obtained with Sephadex G-25, which has an exclusion limit at a molecular weight of about 4000, the molecular weight of thrombin probably is higher than 4000 These findings are in accordance with Cohley *et al* (1961) who found a molecular weight of about 7000 for the thrombin monomer by ultracentrifugation A similar figure was obtained by Laki *et al* (1964) who analysed the aminoacid sequences Magnusson (1965 c), however, did not obtain these results

The application of gel filtrated thrombin on a CM-Sephadex column for further purification was performed at an ionic strength of 0 1 M It would be of great advantage to perform the gel filtration also at this ionic strength However, there was a marked adsorption to the Sephadex With Sephadex G-25, the adsorption was almost complete This adsorption phenomenon can be used for preparation because the thrombin can be eluated with 0 5 M NaCl However this procedure does not seem to have any advantage compared with the use of CM Sephadex

The adsorption phenomenon may explain why thrombin can be separated from protein of greater molecular weight by means of Sephadex G 50 At an ionic strength of 0 5 M, there may be a retardation of the molecules during the passage of thrombin through the Sephadex column which may give the impression that a small molecule is involved

Further purification and concentration of thrombin on a CM-Sephadex column

The chromatography of thrombin on ion exchangers has been commonly used The cationic exchanger Amberlite IRC-50 was used by Rasmussen (1955) while Miller (1959), Miller and Copeland (1962), and Seegers (1962) used IRC-50-Rivanol Phosphate cellulose was used by Seegers and Landaburu (1960) and DEAE-cellulose by Magnusson (1965 a) Cationic exchangers such as CM-cellulose absorb thrombin, but Seegers *et al* (1960) and Seegers (1962) found that activity was lost in the process of chromatography A short CM-cellulose column was used by Korsan-Bengtsen and Ygge (1961), which seemed to minimize the loss of activity

The thrombin obtained from the gel filtration was dialysed against 0 1 M Tris-HCl, pH 7 5 and the whole bulk was applied on a short column of CM-Sephadex All the thrombin was ad sorbed but some inert protein passed through In the next step more inert protein was eluted with 0 3 M Tris-HCl pH 8 The thrombin was eluated with 0 5 M NaCl-Tris HCl, pH 8 With 1 M NaCl, an additional small amount of inert protein was eluted, but no thrombin could be found

One great advantage in using CM-Sephadex to separate plasminogen from

thrombin is that plasminogen does not adsorb to CM Sephadex at this ionic strength and pH

A short column was used because the yield of thrombin activity decreases on a long column The thrombin from the gel filtration was concentrated in this manner as the adsorption capacity of CM Sephadex is great

The thrombin was stored at +2 to +4° C because freeze-drying and freezing decrease the activity The final preparation contained about 30 000 NIH units/mg tyrosine and thus was among the preparations with the highest activity Magnusson (1965) obtained *2100 NIH units/mg protein (which is* about 20 300 NIH units/mg tyrosine) Seegers (1962) obtained 45 000 NIH units/mg tyrosine An activity as high as 99 400 Iowa units (which is about 59 000 NIH units* /mg tyrosine) was obtained by Miller and Copeland (1962)

The preparation obtained from the CM Sephadex chromatography contained about 600 NIH *units/ml In spite* of this high thrombin concentration no fibrinolytic activity was observed if crude thrombin from the $BaSO_4$ adsorbed plasma was used as a starting material However when Topostasine which contains more plasminogen was used traces of fibrinolytic activity still remained in the final preparation Re-chromatography or re filtration on Sephadex G 75 may possibly have separated *these last plasminogen traces from* Topostasine Such experiments were not performed however

III Preparation of human plasminogen suitable as substrate for quantitative determination of plasminogen activators

There is an extensive amount of literature describing the preparation of plasminogen A review has been made by Wallen (1962 a) Bergstrom (1963 b) and MacGay (1964) Only a short survey of previous works which have connection with the method described in Paper III will be presented here

If plasminogen is to be used as a substrate in a quantitative determination of urokinase it is essential that the plasminogen preparation is soluble at physiological ionic strength and at neutral pH and that it contains only minimal plasmin amounts

Plasminogen has usually been prepared from Cohn s Fraction III by means of precipitation with strong mineral acids and alkali according to the method of Kline (1953) This plasminogen is insoluble and unstable at neutral pH and physiological ionic strength However addition of l lysine and ε aminocaproic acid increases the solubility These substances have been used in the preparation procedure by Alkjaer s g Fletcher and Sherry (1959) Wallen and Bergstrom (1960) Wallén (1962 b) and Hagan *et al* (1960) Derechin (1961) prepared plasminogen that was soluble at neutral pH from outdated human plasma Mineral acid was avoided

* The tyrosine content 10 4% (Miller *et al* 1962)
** 0 60 NIH units 1 00 Iowa unit (Magnusson 1965)

Slotta, Michl, and Santos (1962) prepared plasminogen from Cohn's Fraction III by means of extraction with lysine buffer at pH 9 and gel filtration on Sephadex G-25 This plasminogen, which they called 'Native', was easily soluble in the physiological pH range Slotta *et al* (1962) and Slotta and Gonzales (1964) have pointed out that this 'Native' plasminogen was different from the plasminogen prepared with strong mineral acids and strong alkali Alkjaersig (1964) prepared plasminogen from Cohn's Fraction III and euglobulin with acid extraction and ε-aminocaproic acid without the use of mineral acids Further purification was performed by means of DEAE-cellulose or DEAE-Sephadex The plasminogen exposed to mineral acids differed in solubility and stability from the plasminogen prepared by means of ε-aminocaproic acid which increased the solubility and stability The preparation also exhibited differences in their ultracentrifugal and electrophoretic behaviour

In the method described in Paper III, the euglobulin prepared from fresh human plasma was used as a starting material The purification was performed by means of gel filtration on Sephadex G-200 and chromatography on DEAE-Sephadex

Euglobulin precipitate as starting material

Cohn's Fractions II and III are commonly used as a starting material for plasminogen purification Alcohol precipitation may result in denaturation of plasminogen To obtain a plasminogen as 'native' as possible, we preferred to use an isoelectric euglobulin precipitate from plasma as starting material Euglobulin precipitation has previously been used by Milstone (1941) and Alkjaersig (1964)

Plasma from healthy blood donnors was chosen The spontaneous fibrinolytic activity, determined by means of euglobulin lysis time, is generally low in normal human blood The plasma was left for 30 minutes at +37° C before the preparation was started The spontaneous, fibrinolytic activity of the final preparation seemed to be low when this procedure was used, possibly because of inactivation of plasmin $BaSO_4$ was added to the plasma to absorb prothrombin which was later used for thrombin preparation (II) Euglobulin was then precipitated from the plasma by diluting the plasma with 19 parts of distilled water and by adjusting the pH to 5 3 with acetic acid

Gel filtration of euglobulin on Sephadex G-200

Gel filtration of highly purified plasminogen on Sephadex G-200 has been performed by Wallen (1962 c) Two peaks which both contained fibrinolytic activity were obtained Slotta, Michl, and Santos (1962) used Sephadex G-25 A separation from small molecules was obtained A plasminogen prepared according to a modified method of Kline was separated by Robbins and Summaria (1963) on Sephadex G-50 and G-75 column equilibrated with 0 0005 N HCl

pH 3 5 Two peaks were obtained The second peak contained plasminogen

Lewis (1964) performed gel filtration of Cohn's Fraction I and obtained a good separation of plasminogen from fibrinogen on Sephadex G-200 He also performed gel filtration of plasma on Sephadex G-100 and G-75 equilibrated with NaCl-Tris buffer pH 8 but minimal or no separation of plasminogen from proteins with larger molecules than those of plaminogen was observed

Shulmann, Alkjaersig, and Sherry (1958) have found the molecular weight of human plasminogen to be 143,000 Davies and Englert (1960) reported a molecular weight of 83,000 which is in agreement with the findings of Bergstrom (1963 a) in bovine plasminogen Because of the relatively high molecular weight of plasminogen, separation from greater molecules than those of plasminogen could not be expected with Sephadex G-75 and G 50

Sephadex G-200, which has an exclusion limit at a molecular weight of 200 000 seemed to be best suited for separation of plasminogen and was therefore chosen in the present experiments

Gel filtration of euglobulin on Sephadex G 200 in the salt buffer solution 0 5 M NaCl and 0 1 M Tris HCl (4 1) pH 8 gave four peaks The plasminogen, however was distributed over a rather wide range The three last peaks contained plasminogen Despite this wide range of plasminogen a separation from the main part of the inert protein was obtained because the major protein peak contained no plasminogen

The wide plasminogen distribution was seen also in the purification of thrombin (II) and is possibly caused by an elongated molecule Adsorption to other proteins may also be an explanation Increasing the ionic strength to 1 M NaCl did not give better results The same plasminogen distribution was obtained by Lewis (1964)

Further purification of plasminogen on DEAE-Sephadex

The chromatography of plasminogen on ion exchangers has previously been done on DEAE-cellulose (Alkjaersig 1960, Derechin 1962, Wallén and Bergstrom 1959, 1960, Slotta and Gonzales 1964) CM-cellulose has also been used by Hagan, Ablondi, and DeRenzo (1960), and by Wallén *et al* (1959) DEAE-cellulose has been used by Wallén (1962 c)

The anionic exchanger DEAE-Sephadex has previously been used by Robbins and Summaria (1963) and Robbins, Summaria, Elwyn and Barlow (1965) as well as Alkjaersig (1960 and 1964) According to Robbins *et al* (1963) plasminogen was adsorbed at pH 8 to 9 in a Tris-lysine buffer with an ionic strength of 0 07 and eluted by increasing the ionic strength of the buffer to 0 17 with NaCl The lysine in the buffer was considered essential

In the present experiments, the eluate from the gel filtration containing plasminogen was divided into two parts Each of them, after dialysing against 0 1 M Tris, pH 8, was chromatographed separately on the DEAE-Sephadex co

lumn The first part contained more protein than the second The plasminogen content was about the same in both A small column of Sephadex (30×70 mm) which was equilibrated with 0 1 M Tris, pH 8 was used At the application all the plasminogen and all the protein in the second part were adsorbed to the column, while some inert protein in the first part passed through

The elution was performed in two steps In the first step the plasminogen was eluted with 0 3 M Tris-HCl, pH 8 The plasminogen activity and the protein amount were almost the same in both parts The second step was performed with a salt-buffer solution of 0 5 M NaCl and 0 1 M Tris-HCl, pH 8 Only inert protein was eluted The plasminogen content in the two parts was almost the same and, consequently, all the eluate from the gel filtration containing plasminogen could be applied on the DEAE-Sephadex column in one step

In addition to being purified, the highly diluted plasminogen from the gel filtration was also concentrated by means of this procedure The final preparation contained about 100 CU/mg tyrosine and was easily soluble at physiological pH and ionic strength

The chromatography was performed without using ε-aminocaproic acid or l-lysine in the buffer A small column and stepwise chromatography gave the best results and were most practical A large column and gradient elution was tried but without success The plasminogen came in several peaks The plasminogen from the experiment above was re chromatographed on a large column and with a gradient A further separation seemed to occur, but the plasminogen activity decreased

Additional properties of the plasminogen preparation*

The purpose of the work described in Paper III was to develop a simple method for preparation of a plasminogen with a high solubility and a low spontaneous activity (plasmin)

The plasminogen preparation was highly soluble as pointed out before Lyophilization decreased the activity only slightly and the solubility did not change The activity of the lyophilized preparation which was stored at —20°C did not decrease for several months However, lyophilization in some way changed the properties of the plasminogen because freeze-dried plasminogen could not be eluted from the DEAE-Sephadex at 0 3 M Tris-HCl

To obtain information about the purity of the preparation, gel electrophoresis and immunoelectrophoresis have been performed Moving boundary electrophoresis of a highly purified preparation has previously been performed by Sgouris, Inman, and McCall (1960) They identified two components The faster component constituted 75—80 % of the total and was thought to represent the plasminogen Slotta *et al* (1962) found three components in Kline's plas-

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* The author is grateful to Doc Bengt Johansson Division of Clinical Chemistry, Psychiatric Research Department St Lars Sjukhus Lund for the performance of the gel electrophoresis and immunoelectrophoresis



Fig 1 Agaroselectrophoresis of plasminogen to the left To the right the *fibrin urokinase* slide which shows the lysis zone of fibrin

minogen In their 'native plasminogen, seven components were observed Robbins and Summaria (1963) found the plasminogen (prepared by a modified method of Kline) to be homogenous in ultracentrifugation But electrophoretic analysis revealed two components Immunoelectrophoresis and gel diffusion showed one component Two components were also found by Shulman *et al* (1958) with electrophoresis and by Hagan *et al* (1960) who used *immunoelectrophoresis* of rabbit *antiplasminogen serum* Wallén (1962 c), in his highly purified preparation, identified two components, both containing plasminogen and moving very near each other if starch gel electrophoresis was performed with ε aminocaproic acid acetate buffer, pH 4 A single component was seen at pH 2 1

The plasminogen obtained by the method described in Paper III was analysed by means of agaros electrophoresis and immunoelectrophoresis against anti human serum

Methods

Agaros electrophoresis was performed according to the method of Wieme (1959) with one modification Agaros (Behringswerke) was used



Fig 2 Immunoelectrophoresis of plasminogen against anti human serum

Identification of plasminogen in the agaros electrophoresis was done with slides covered with a film of agar containing fibrinogen and urokinase These slides were prepared as follows

1 ml plasminogen free fibrinogen (0 2 %) was mixed with 0 2 ml urokinase, 100 Ploug units/ml 0 5 ml 2 % agar was added to the mixture melted and warmed to +50° C 0 2 ml thrombin 10 NIH units/ml, was added to the agar Immediately after mixing the solution was poured on glass slides of the same size as those used for electrophoresis The slides were incubated at +37° C for 2 hours and then stored at +4° C until use

When the electrophoresis was completed the plasminogen was identified by placing the fibrin urokinase slides over the electrophoresis slides and incubating for 30 minutes at +37° C Then the fibrin urokinase slide was removed and the fibrin was stained A clear zone was seen where the formed plasmin had digested the fibrin

Immunoelectrophoresis of the plasminogen was performed against anti human serum The method of Scheidegger (1955) modified by Clausen Dencker, and Svennerholm (1964) was used Before application the plasminogen was concentrated three times

The plasminogen preparation used was that obtained from DEAE Sephadex The activity of the preparation was about 30 CU/ml

Result

In the agaros electrophoresis, two main components were seen (cf Fig 1) The first (a) had the same moving velocity as transferrin which was used as reference substance The other component (b) was smaller and is scarcely reproduced in the figure On the slide, still two trace components which could not be reproduced in the figure could be seen in front of the main component On the fibrin urokinase slide, there was a

rather broad clearness which belonged to the main component and possibly also to the component behind this

The immunoelectrophoresis is shown in Fig 2 Small precipitate lines can be observed in the α—β region The lines possibly represent impurities No attempt was made to identify the lines

Accordingly, the preparation was not homogenous The finding of two components in the agaros electrophoresis agrees with the results of other authors According to the immunoelectrophoresis, there were traces of several impurities In spite of the simplicity of the preparation method, it is comparable with the best preparations previously made as far as impurities are concerned

PART TWO

Kinetic and Methodological Studies

IV Plasminogen assay by means of the lysis time method

The quantitative determination of plasminogen is based on the measurement of plasmin, the activated form of plasminogen As plasmin splits not only fibrin but also some other proteins and synthetic esters, a great number of substrates have been used to determine plasmin The most commonly used substrates have been fibrin (Loomis George, and Ryder 1949, Astrup and Mullertz 1952) casein (Remmert and Cohen 1949) and synthetic esters (Troll, Sherry, and Wachman 1954, Roberts 1958)

The common methods with fibrin as substrate are the fibrin plate method (Astrup *et al* 1952) and the methods based on clot lysis (the lysis time method)

In the commonly used lysis time method plasminogen is activated into plasmin by means of streptokinase or urokinase in a first stage In a second stage, fibrinogen and thrombin are added and a fibrin clot is formed in the presence of plasmin The plasmin lyses the formed fibrin, and the time for complete lysis of the clot (lysis time, T) depend on the amount of plasmin present in the clot

Several methods in which the plasmin activity is determined by means of the lysis of a preformed clot have been described However, the formation of the clot in the presence of plasmin is followed by a much more rapid lysis, as compared with the clot which is formed before plasmin is added

Recording the end-point of the clot lysis

A visual observation of the end point of the fibrin lysis can be difficult and several methods have been worked out to increase the accuracy of the determination of the clot lysis The test tube has been shaken to obtain air bubbles in the clot which disappear when the clot is lysed (Ambrus 1960) Glass beads have been placed on top of the clot and the time required for the beads to reach the bottom has been used as a measure of the clot lysis (Kjeldgaard and Ploug 1957 a) Determination of substances soluble in trichloroacetic acid obtained by lysis of fibrin or fibrinogen after a certain time has been used by Bergstrom and Wallen (1961), Mosesson and Fin-

laysson (1963) and Mackay (1964) Determination of the opacity of the clot has also been used (Pennel 1960) Astrup and Egeblad (1965) and Egeblad (1966) have used thrombelastography However, direct observation of the disappearance of the fibrin is commonly used and gives a high degree of accuracy by a skilled person

We have investigated the accuracy of the observation of the clot lysis on the part of two of our laboratory assistants who read the lysis time of ten different plasminogen amounts each of which was activated with the same urokinase amount To avoid the influence of the decay of urokinase or plasminogen during the investigation the assistants performed the same assay approximately at the same time The watch glasses were covered to avoid the influence of previous tests and of each other Double determinations were made The results are shown in Table 1

Table 1 Lysis time assays performed by two laboratory assistants Lysis time is expressed in seconds

Sample	Assistant 1		Assistant 2	
1	[illegible]	[illegible]	207	207
2	353	343	305	305
3	384	395	389	394
4	[illegible]	570	549	530
5	543	555	546	545
6	696	696	683	695
7	688	715	707	709
8	955	975	935	967
9	[illegible]	[illegible]	984	939
10	1335	[illegible]	1319	1323

The standard deviation (s) was calculated according to the formula

$$S = \sqrt{\frac{\sum (x_1 - x_2)^2}{2n}}$$

where $\sum (x_1 - x_2)$ is the sum of the difference between each pair of duplicates and n the number of duplicates The standard deviation was ±8 5 sec The lysis time of the samples varied from 200 sec to 1330 sec

A comparison between the precision of the two laboratory assistants was made The standard deviation of the average difference from zero was calculated according to the Students t test

$$t = \frac{\bar{d} - o}{S_d} \sqrt{n}$$

where $\bar{d}$ is the average difference between the two assistants and S_d is the corresponding standard deviation n is the number of duplicates t was found to be 0 94 The value for t at the 5 per cent level was 2 26 at 9 degrees of freedom Thus no significant difference between the assistants was observed

Activation of plasminogen

As previously pointed out the determination of plasminogen by means of a two stage assay in which the formed plasmin is incorporated into the clot involves the following reactions

In the first stage activation of plasminogen into plasmin

In the second stage formation of fibrin from fibrinogen and degradation of the formed fibrin by means of the plasmin formed in the first stage

The activation of plasminogen into plasmin is performed as a separate reaction Streptokinase is commonly used as activator However, the maximal plasmin activity obtained from a certain plasminogen concentration depends on the streptokinase amount used Besides activating plasminogen, streptokinase also inactivates the formed plasmin (Blix 1961)

The investigations presented in Paper IV verify that the maximal plasmin activity is obtained only with a certain streptokinase concentration There is a relationship between the maximal plasmin activity and the streptokinase amount necessary to obtain this activity If this optimal streptokinase concentration is exceeded, a lower activity is obtained

Different forms of streptokinase-activated plasminogen have been suggested by Markus and Ambrus (1960 a) and, according to Markus and Werkheiser (1964), a small streptokinase amount activates plasminogen into plasmin enzymatically This plasmin is later bound stoichiometrically to streptokinase in a streptokinase-plasmin complex which has a lower fibrinolytic and caseinolytic activity, but which still has maximal TAME activity This complex also has activator activity for bovine plasminogen

High urokinase concentrations do not inhibit plasmin Thus urokinase ought to be preferred as activator The plasmin activity increases with the urokinase concentration and very high concentrations are necessary to obtain maximal plasmin activity at +37° C In purified systems, it is possible to obtain a higher activity with urokinase than with streptokinase

As can be expected from an enzymatic reaction, Kjeldgaard and Ploug (1957) obtained the same final plasmin activity with various urokinase amounts if casein was present in the reaction mixture and if the reaction was performed at +4° C Without casein we found various levels of plasmin activity for the first 5 hours if suboptimal urokinase concentrations were used This will be further discussed in Paper VI

With streptokinase concentrations and with urokinase, the activation is rapid and maximal plasmin activity is obtained within the first five minutes If longer activation times are used the inactivation will be pronounced

Formation of fibrin

The plasmin formed in the first stage is determined in the second stage This stage consists of the clot formation and the clot lysis

The formation of fibrin from fibrinogen by means of thrombin has been studied extensively in the last decade It is assumed that four polypeptides, two fibrinopeptides A and two fibrinopeptides B, are split off by thrombin and that a polymerization is started The various steps of the fibrinogen fibrin conversion have been studied by Blomback (1958), Blomback and Laurent (1958), Scheraga and Ehrenpreis (1958), Laki and Gladner (1964) and others Reviews have been made by Scheraga *et al* (1958) and Laki *et al* (1964)

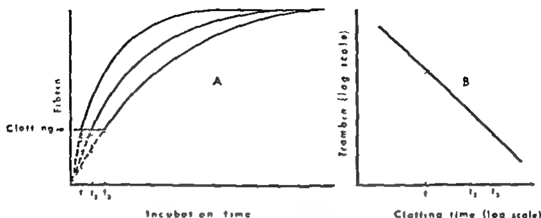


Fig 3 The initial velocity of fibrin formation determined by clotting time A shows the fibrin formation plotted against time for three thrombin concentrations B shows the relationship between the thrombin concentration and the clotting time

The rate of the over-all reaction of the fibrin formation might influence the reaction of the clot lysis The fibrin formation has been studied by determining the fibrin amount at intervals Formaldehyde was used to stop the polymerization according to a method described by Scheraga *et al* (1958) It was found that the over-all reaction of the fibrin formation at constant thrombin follows a simple, first-order reaction with respect to time As pointed out by Scheraga *et al* (1958), however, this reaction is in fact very complicated

A study of the fibrin formation with two different thrombin concentrations showed that the rate increased with increasing thrombin concentration The relationship between the initial velocity and the thrombin concentration can be estimated by determining the clotting time It can be assumed that at constant fibrinogen concentration, the same fibrin amount is necessary to form a visible clot and that the clotting time will depend on the thrombin concentration If the fibrinogen concentration is not too low, the clotting time expresses the initial velocity which is schematically shown in Fig 3 A

$$v = \frac{Fc}{Tc} \qquad (1)$$

where v is the initial velocity and Fc is the fibrin concentration at the clotting time (Tc) If the clotting time is plotted against the thrombin concentration, the middle of the curve approximately follows the equation (Seegers 1962)

$$\frac{Fc}{Tc} = kE \qquad (2)$$

where E is the thrombin concentration and k the rate constant On a log x log graph, a straight line is obtained which is schematically shown in Fig 3 B In

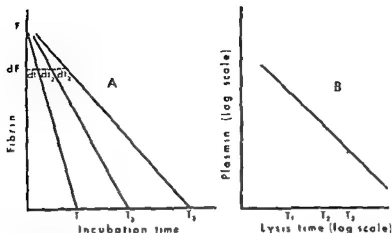


Fig 4 The initial velocity of fibrinolysis determined by the lysis time A shows how the initial fibrin degradation is proportional to the lysis time B shows the relationship between lysis time and plasmin concentration

this range of thrombin concentrations, the rate of fibrin formation will be

$$\frac{dF}{dt} = v = \frac{Fc}{Tc} = kE \quad (3)$$

Thus, the formation approximately follows a first-order reaction with respect to thrombin At higher thrombin concentrations, a deviation from this simple equation is seen (Seegers 1962) However, if a high thrombin concentration is used, this reaction is so rapid that the reaction is of little importance for the over all reaction which is pointed out below

Lysis of the fibrin

The formed plasmin lyses the fibrin clot and the time for complete lysis depends on the plasmin amount The rate of the spontaneous disappearance of fibrin formed from plasma has been studied by Fernley (1960) and in a purified system by Pennel (1960) Both determined the opacity of the clot The disappearance was not linear After liquefaction of the fibrin, lowering of the temperature to stop the reaction, and precipitation, Mosesson and Finlayson (1963) found that the increase in absorbance of light at 280 mμ of the supernatant fluid was linear with time until about 10 % of the initial clottable protein remained This is in accordance with our findings with respect to the release of perchloric acid soluble substances from lysis of a fibrin clot, which was determined by means of increase of absorbance at 280 mμ There was a linear relationship with time which continued after the clot was lysed The decrease in the fibrin amount in the clot was also studied by measuring the amount of remaining fibrin at intervals To avoid degradation of the fibrin

during the determination, the reaction was stopped with formaldehyde A control experiment was performed which showed that formaldehyde added to the clot immediately stopped the action of plasmin ε-aminocaproic acid was also tried but without success The decrease in fibrin was found to be approximately linear with respect to time and, accordingly, of zero order

The over-all reaction

The over-all reaction of the formation and the degradation of fibrin is the result of two consecutive reactions The formation follows a first order reaction while the degradation follows a zero-order reaction with respect to time The equation of the over-all reaction at constant thrombin and plasmin will be

$$F = F_0 (1 - e^{-k_1Et}) - k_2Pt \quad \text{(IV 6)}$$

where F is the fibrin amount at the time t F_0 is the initial fibrinogen concentration E is thrombin, and P is plasmin k_1 and k_2 are rate constants If the thrombin concentration is high, the coagulation will be rapid and e^{-k_1Et} will be negligible Thus the equation for the over-all reaction will be

$$F = F_0 - k_2Pt \quad \text{(IV 7)}$$

The course of the over-all reaction has been studied in an experiment in which a high and a low thrombin concentration were used With the high thrombin concentration, practically the whole course of the reaction followed the zero order However, the lysis time was almost the same with the low as with the high thrombin concentration This was not expected The cause was possibly that, besides a fibrinolysis, a fibrinogenolysis occurred which had approximately the same rate

The relationship between the lysis time and the plasmin concentration

The relationship between the initial reaction rate of the fibrinolysis and the plasmin concentrations can be obtained by determining the lysis time at various plasmin concentrations which is schematically shown in Fig 4 If the degradation is of zero order with respect to time and a high thrombin concentration is used, the over-all reaction follows equation (IV 7) The initial reaction rate (v) is (Fig 4)

$$-v = -\frac{dF}{dt} = -\frac{F_0}{T} \quad (4)$$

where F_0 is the initial fibrinogen concentration and T the lysis time The relationship between the lysis time (T) and the plasmin concentration (P) has experimentally been shown to be

$$\frac{1}{T} = \frac{kP}{F_0} \quad \text{(IV 8)}$$

within a certain region Accordingly

$$-v = -\frac{dF}{dt} = kP \quad (5)$$

The rate of fibrin lysis is proportional to the plasmin concentration

Kjeldgaard and Ploug (1957 b) found a linear relationship between the inverse of the lysis time and the plasmin concentration Astrup and Egeblad (1965) found a linear relationship between the

lysis time and the plasmin concentration in the log x log scale The slope of the line was about —1 5 Thrombelastography was used to determine the lysis time

In the present experiments, there was an inverse proportionality between the plasmin concentration (P) and the lysis time (T) according to the equation

$$T = \frac{F_0}{k_-} \cdot \frac{1}{P} \qquad \text{(IV 8)}$$

or

$$\log T = - \log P + \log \frac{F_0}{k_?} \qquad \text{(IV 9)}$$

The logarithmic form of this equation shows that there is a linear relationship between log of the lysis time and log of the plasmin concentration The slope of the line is —1 This relationship is valid only within a certain part of the line At high plasmin concentrations, the lysis of the clot is difficult to observe accurately At low plasmin concentrations, an inactivation occurs which increases the lysis time If inactivation occurs, the slope will be greater than —1, which is seen for instance if the streptokinase concentrations are too high If activation occurs, the slope will be less than —1 which is shown in Paper VII

The findings of Astrup *et al* (1965) are difficult to compare with our results as thrombelastography and direct observation of lysis are very different methods

Significance of the fibrin concentration

According to equation (IV 8) the lysis time is proportional to the fibrinogen concentration while, according to equation (IV 9), the fibrin concentration (F_0) determines the distance of the line from origin At constant plasmin the lysis time increases linearly with the fibrinogen concentration Thus, smaller plasminogen amounts can be determined with greater accuracy when a low fibrinogen concentration is used The concentration, however, cannot be lower than 0 04 %, otherwise the clot will be so tiny that observation of the lysis will be difficult

The assay

As previously mentioned, the assay is performed as a two stage method In the first stage the plasminogen is activated with either streptokinase or urokinase If streptokinase is used, an optimal concentration giving maximal activation must be found

After maximal activation, thrombin and fibrinogen are added The thrombin is added first to avoid fibrinogenolysis A high thrombin concentration and a low fibrinogen concentration are used The lysis time is recorded and the plasmin concentration is read from a standard curve

The standard curve of the assay is easy to construct as the slope of the curve is —1 if log of the lysis time is plotted against log of the plasmin concentration It is sufficient to know one plasmin concentration and its lysis time As casein units are most commonly used the plasminogen concentrations are expressed in these units

In conclusion, it can be said

Direct observation of the clot lysis can be performed with great accuracy by trained persons There is an inverse proportionality between the lysis time and the plasmin concentration The standard curve is easy to construct as the slope is —1 in the log x log scale This relationship is valid only within a certain range of plasminogen concentrations The fibrinogen concentration is an important factor Low concentrations give shorter lysis time and higher sensitivity

If streptokinase is used as activator, there is a certain relationship between the plasminogen concentration and the optimal streptokinase concentration Inactivation occurs if this optimal concentration is exceeded Urokinase gives a slightly higher activity while high concentrations do not inactivate the plasmin formed

V A method to correct for the continuing activation during the second stage in a two-stage assay Exemplified by urokinase activation of plasminogen determined by the lysis time method

The common principle to determine the total amount of an inactive precursor of an enzyme by means of a two-stage method is to completely activate the precursor in the first stage and then determine the activated enzyme in the second stage Plasminogen, e g , is completely activated by urokinase or streptokinase in a first stage Then the plasmin formed is determined in a second stage as described in Paper IV

However, it is difficult to follow the course of a reaction by this method In many of the quantitative two-stage methods used in coagulation and fibrinolysis, all the reacting substances are proteins and there are no simple methods to stop the reaction in the first stage without inactivating the product formed or the substrate left It is difficult, e g , to follow the course of the urokinase activation of plasminogen into plasmin by means of the lysis time method If determination of the plasmin formed is performed before complete activation, the reaction will continue in the second stage during lysis of the fibrin clot Thus, both the plasmin formed in the first stage and that formed in the second stage are recorded during the determination

If the data obtained from a kinetic study of a reaction are plotted according to the usual formulae for different orders of reactions, there will be a deviation from the usual graphs and the amount of product at any time cannot be calculated With respect to fibrinolysis, attempts have been made to stop the reaction before the determination In activation experiments with urokinase and plasminogen, Alkjaersig, Fletcher, and Sherry (1958) stopped the reaction by precipitating the formed plasmin with 1 M NaCl The urokinase remained in the supernatant This method was unsuccessful in the present experiments The reason for this may be that the

above authors used plasminogen prepared according to a modified method of Kline (1953), which possibly is denaturated and has other physical properties With the plasminogen prepared according to our method (cf Paper III) the plasmin was inactivated

According to Kjeldgaard and Ploug (1957) 'fast methods such as the lysis time method · minimize the activation during the determination However, it is clearly shown in the experiment described in Paper V, which deals with urokinase activation of plasminogen, that a considerable activation occurs during the determination

The formulae calculated in Paper V take into account the activation which continues during the determination Thus, it is not necessary to stop the reaction If the reaction follows any of the different orders given in Paper V, it is possible to calculate the plasmin amount formed until the moment the determination is started No such method has previously been described, at least not with in the fields of coagulation and fibrinolysis

The mathematical procedure

To simplify the calculation, it is assumed that the reaction follows the same order in the first stage as in the second stage Certainly this may not always be the case The rate equation during the determination in the second stage may be more complicated However, if the determination does not last too long the error will be minimal if the reaction is approximated to the same order within both stages

The mathematical development has been based on the urokinase activation of plasminogen The rate equation was integrated within two intervals first, from zero incubation time until the determination was started (the first stage), secondly, during the determination (the second stage) The first equation is substituted in the second and the following equation is obtained

$$\ln P_m - \ln (P_m - P_T) = k_1 U t + k_2 U T \qquad (V\ 5)$$

where P_m is the maximal plasmin activity and P_T is the recorded activity t is the reaction time and T the duration of the determination = lysis time U is the urokinase concentration k_1 is the rate constant of the reaction in the first stage and k_2 is the rate constant in the second stage The presence of the clot in the second stage will change the rate constant This has also been shown experimentally An expression for the rate constant (k_o) in the second stage is obtained from equation (V 5) if the incubation time is zero

$$k_2 = \frac{\ln P_m - \ln (P_m - P_{t_0})}{U T_{t_0}} \qquad (V\ 7)$$

where T_{t_0} is the duration of the second stage (lysis time) at zero incubation time P_{t_0} is the recorded data at $t = 0$ In practice this is performed by doing the test as a one-stage assay When this is done, the activation proceeds only during the determination (second stage)

If k_o is substituted in equation (V 5), the formula for a first-order reaction will be obtained

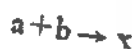
The formulae

Formulae have been calculated for reactions of first, second and zero order reactions The formula for a first order reaction has the following form

$$-\ln\left(1-\frac{P_t}{P_m}\right) = -\ln\left(1-\frac{P_t}{P_m}\right) + \frac{T}{T_{to}}\ln\left(1-\frac{P_{to}}{P_m}\right) = k_1Ut = A \qquad (V\ 9)$$

where P_t is the activity at the time t when the second stage is started and P_T is the activity recorded when the reaction in the first stage has proceeded for the time t T is the duration of the determination in the second stage (lysis time) P_{to} is the activity recorded at zero incubation time and T_{to} is the duration of the determination when the determination is done at zero incubation time, i e when the whole reaction proceeds only during the determination or when a one-stage assay is performed k_1 is the rate constant

If a second-order reaction goes according to the stoichiometric formula



$$a + b \rightarrow x$$

the formula is

$$\ln\frac{(b-x_t)\,a}{(a-x_t)\,b} = \ln\frac{(b-x_T)\,a}{(a-x_T)\,b} - \frac{T}{T_{to}}\ln\frac{(b-x_{to})\,a}{(a-x_{to})\,b} = k(b-a)\,t = A \qquad (V\ 14)$$

x_t is the activity of the product obtained at the time t and x_T the activity recorded x_T also contains the activity formed during the determination x_{to} is the activity recorded at zero incubation time a and b are the maximal activities

For a zero-order reaction the following formula is obtained

$$a_t = a_T - \frac{T}{T_{to}}\,a_{to} = kt \qquad (V\ 16)$$

In principle, all the formulae have the same form They consist of the difference of two expressions The first is the usual formulae containing the recorded data The second expression is a correction factor containing the data recorded at zero incubation time multiplied by the ratio of the duration of the determination at the time t and the duration at zero incubation time

If the expression for a first order reaction is called A according to equation (V 9)

$$-\ln\left(1-\frac{P_t}{P_m}\right) = A \qquad (V\ 9)$$

the activity at the moment the determination starts (P_t) can be calculated according to the following equation

$$P_t = P_m\left(1 - e^{-A}\right) \qquad (V\ 10)$$

At zero incubation time $P = P_{to}$ and $T = T_{to}$ which substituted in equation (V 9) gives $A = 0$ and then $P_t = 0$ If the reaction approaches completion, $A \rightarrow -\infty$ and then $P_t \rightarrow P_m$ The other formulae can be analysed in a corresponding manner

The use of the formulae in fibrinolysis

If plasminogen is activated with urokinase and the plasmin formed is deter-

mined by means of the lysis time method, the relationship between the plasmin activity and the lysis time is expressed by the following equation

$$P = \frac{F}{k_3 T} \qquad \text{(IV 8)}$$

where P is the plasmin activity, F the fibrinogen, T the lysis time and k_3 the rate constant If the various plasmin activities are expressed by means of their lysis time and substituted in (V 9) the following equation is obtained

$$-\ln\left(1 - \frac{T_m}{T_t}\right) = -\ln\left(1 - \frac{T_m}{T}\right) + \frac{T}{T_{to}} \ln\left(1 - \frac{T_m}{T_{to}}\right) = k_1 Ut = A \qquad \text{(V 12)}$$

where T is the lysis time recorded after the incubation time t and T_t is the lysis time if the reaction could be stopped at this moment T_m is the lysis time of maximal activity obtained with the urokinase U

If the reaction follows a first-order reaction with respect to time a straight line from origin will be seen if A is plotted against time The slope of the line is the rate constant multiplied by the urokinase concentration (VI)

The plasmin activity at the time the determination is started can be calculated according to the formula

$$\frac{1}{T_t} = \frac{1 - e^{-A}}{T_m} \qquad \text{(V 13)}$$

or expressed as P_t

$$P_t = \frac{F(1 - e^{-A})}{k_3 T_m}$$

Experimental verification

An experiment in which plasminogen was activated with urokinase in a first stage was performed At time intervals, aliquots were taken from the reaction mixture and the plasmin formed was determined by means of the lysis time method in a second stage The activation was performed at +15° C at which temperature the inactivation is minimal The determination of the plasmin formed was done at +37° C If the recorded data are plotted against the reaction time, the curve does not start at origin As early as at zero incubation time, there is a fibrinolytic activity which represents the activation during the determination in the second stage If the recorded data, corrected according to formula (V 13), are plotted in the same manner the curve starts at origin The two curves approach each other and join at the maximal activity (Fig V 1) If A is calculated according to the formula above and is plotted against the reaction time, a straight line is obtained which indicates a first-order reaction with respect to time (Fig V 2)

The rate constant

The rate constant (k_1) before and during the determination (k_2) is not the same This has been shown experimentally An expression for k_2 was obtained from equation (V 7) which can be written

$$\ln \frac{P_m}{P_m - P_{to}} = k_2 U T_{to}$$

or if the lysis time is substituted

$$-\ln\left(1-\frac{T_m}{T_{t0}}\right) = k_2\, UT_{t0} \quad (V\ 18)$$

Various plasminogen amounts were activated with a fixed amount of urokinase Then the plasmin activity was determined by means of the lysis time method at zero incubation time, i e the test was performed as a one-stage assay If $-\ln\left(1-\frac{T_m}{T_{t0}}\right)$ was plotted against T_{t0} a straight line was obtained The slope of the line was an expression for the rate constant of the reaction during the determination (k_2)

The rate constant of the reaction before the determination was started could be obtained from the formula for A (V 12) which, plotted against the reaction time, gave a straight line The slope of the line was an expression for the rate constant (k_1) These two lines had different slopes The temperature was the same in both experiments One cause of the difference between the two constants is probably the presence of a fibrin network which impedes the collision of the plasminogen and activator molecules

VI Urokinase activation of plasminogen and spontaneous inactivation of the formed plasmin A kinetic study

The urokinase activation of plasminogen has previously been found to follow a first-order enzymatic reaction (Sgouris, Taylor, and McCall 1956, Sherry and Alkjaersig 1956, Alkjaersig, Fletcher, and Sherry 1958, Kjeldgaard and Ploug 1957) Plasmin was found to be highly labile and the activation was performed in the presence of casein (Kjeldgaard *et al* 1957) which protects plasmin from inactivation (Kline 1954) Alkjaersig *et al* (1958) found that glycerol, too, protected plasmin from inactivation Thus, glycerol was used in their experiment with trypsin activation of plasminogen However, the urokinase activation was performed without glycerol because the activation time was so short that minimal inactivation was presumed to take place

When log of the plasmin activities was plotted against time according to a first-order reaction, Kjeldgaard and Ploug (1957) found that the slopes of the lines were proportional to the urokinase concentrations Alkjaersig *et al* (1958) found a linear relationship between the initial reaction velocity and the urokinase concentration A first-order activation kinetics was demonstrated over a wide range of initial plasminogen concentrations If the initial velocities were plotted according to Lineweaver and Burk (1934), a straight line which did not pass through origin was obtained, indicating an enzyme reaction The plasmin activity was determined by means of a caseinolytic assay after stopping the reaction by means of precipitation with 1 M NaCl

Kjeldgaard and Ploug (1957) found the same final activity if a constant plasminogen was activated with various urokinase concentrations, also indicating an enzymatic reaction The activation had to be performed in the presence of a

casein concentration as high as 2.5 % and at a temperature between ±0° and +4° C. The activation was continued for 60 hours. If the casein concentration was lower, the same level was not obtained with the different urokinase concentrations. Sgouris, Taylor, and McCall (1956) also obtained the same activity if the activation was performed at a temperature between ±0° and +2° C without casein. The final level was not reached until 39 days for the lowest urokinase concentration.

Paper VI shows that, after the first hour, different activity levels which are approximately constant in the next 4 hours are obtained. These levels are proportional to the urokinase concentration.

The formed plasmin is sensitive to higher temperatures as inactivation occurs. Then the over all reaction rate is the difference between the rate of activation and of inactivation.

The activation rate

In the investigations described in Paper VI, various plasminogen amounts were activated with a fixed amount of urokinase. These activation studies form the basis for the urokinase determination method which is performed at +37° C (VII). This is the reason why activation has been performed at this temperature although an inactivation which causes a deviation from the first order occurs. However, the influence of the inactivation in the initial part of the reaction is minimal.

The activity of the plasmin formed was determined by means of the lysis time method (IV) and expressed as the reverse of lysis time. The data recorded were corrected for activation of plasminogen during the determination according to the method described in paper V. There was a constant error owing to the use of $\frac{1}{T_m}$ in the formula. Because of the inactivation $\frac{1}{T_m}$ was lower than the maximal activity. However, $\frac{1}{T_m}$ was proportional to the plasminogen concentration. Thus, the relationship between the initial velocities was not influenced. Proportionality was found between the initial reaction rate and the plasminogen concentration which is in agreement with the findings of Kjeldgaard *et al.* (1957) and Alkjaersig *et al.* (1958).

In an enzymatic reaction, the relationship between the initial velocity and the substrate concentration follows a hyperbolic function. At low substrate concentrations the function is approximately rectilinear. At high substrate concentrations, the velocity slows up and approaches a level value, the maximal velocity. This maximal velocity could not be obtained with the urokinase concentration used in this experiment. When the plasminogen concentration was increased further, the plasmin activity became too high to be determined with the lysis time method. When the urokinase concentration was lowered to one Ploug unit/ml a maximal velocity was obtained with 30 CU/ml. Kjeldgaard and Ploug (1957) and Alkjaersig *et al.* (1958) could not obtain maximal velocity with the

high urokinase concentration they used in their experiment. The low solubility of their plasminogen also made the use of such high concentrations impossible.

With respect to the substrate, the activation thus followed a first-order reaction only within a certain range of plasminogen concentrations. At high concentrations, a maximal velocity was reached according to an enzymatic reaction following Michaelis-Menten's principle.

If a fixed plasminogen amount was activated with various urokinase concentrations the initial velocity was proportional to the urokinase concentration within a wide range of concentrations. Thus, the initial rate was of a first order with respect to urokinase which is in agreement with the findings of Kjeldgaard and Ploug (1957) and Alkjaersig *et al.* (1958). When the time course of the reaction was plotted according to a first-order reaction, straight lines were obtained and the slopes of the lines were proportional to the urokinase concentrations which also is in agreement with the findings of Kjeldgaard and Ploug.

The maximal activity

In an enzymatic reaction, the final level of product is independent of the amount of enzyme. According to this, Kjeldgaard and Ploug (1957) found that the plasmin level was independent of the urokinase concentration in the presence of casein. This is in agreement with the findings of Sgouris *et al.* (1956) who did not use protective agents but who performed the activation at still lower temperatures (±0° to +2° C). The final levels were reached after several days.

In the present work an experiment is described in which a fixed plasminogen amount was activated with various urokinase concentrations. The activation was performed at +4° C. Despite this low temperature different activity levels were obtained already in the first hour. During the next 4 hours the levels changed very little. A slow activity increase was seen, however, but still after 4 days different activity levels were observed. Thus, the main part of the activation took place during the first hour, and different activity levels which were proportional to the urokinase amount were obtained. The cause of these different activity levels seen in the experiment described is not known. At +4° C the inactivation is minimal. At higher temperatures than +4° C an inactivation occurs which causes an equilibrium between activation and inactivation. An expression for this equilibrium can be obtained from the rate equation for the over-all reaction of activation and inactivation (VI 7).

$$\frac{dB}{dt} + k_2B = k_1UP_m e^{-k_1Ut}$$

where B is the plasmin activity at the time t, U is the urokinase concentration and P_m the maximal activity if no inactivation occurs. k_1 and k_2 are rate constants. The maximal activity can be obtained by setting $\frac{dB}{dt} = 0$. Then

$$B_{max} = \frac{k_1Ue^{-k_1Ut_{max}}}{k_2} \cdot P_m$$

Accordingly, at constant plasminogen (P_m), B_{max} is dependent on the urokinase concentration (U) and the expression $e^{k_1 U t}$ max in which t_m is the time it takes for the maximal activity to be reached If t_{max} decreases at the *same proportion as U increases this expression* will be constant and the maximal activity will depend on the urokinase concentration

The influence of the temperature on the rate

The activation at different temperatures shows the typical behaviour of an enzymatic reaction The rate increases to a maximum, in this case at +42° C, then it rapidly decreases

The rate equation

The rate of the urokinase activation of plasminogen is in accordance with Michaelis-Menten s principle Thus there is a proportionality both between the initial velocity and the enzyme concentration (urokinase) and between the initial velocity and the substrate concentration (plasminogen) at low substrate concentrations With respect to plasminogen, this is in accordance with a first oder reaction At high plasminogen concentrations the reaction follows a zero order

As pointed out before, the reaction does not go to completion at least not during the first five hours Different activity levels proportional to the urokinase concentration are obtained if a fixed plasminogen amount is activated with various urokinase concentrations

If a low plasminogen concentration is used and if the influence of the inactivation is disregarded the enzymatic equation can be approximated to a first-order reaction with respect to time *Thus, it can be written*

$$-\ln\left(1-\frac{P}{P_m}\right) = Ukt$$

where P_m is the maximal activity obtained with the urokinase U, P the activity of plasmin at the time t, and k the rate constant

The inactivation of the formed plasmin

A mathematical model for the kinetics of the inactivation of enzymes has been set up by Reiner (1959) The kinetics of the inactivation of several enzymes has been investigated (Laidler 1958) The rate of the spontaneous inactivation of plasmin however, has not been studied previously

In the present work, it is shown that the inactivation is enhanced by increasing temperatures Between +4° C and +15° C the inactivation is negligible during the first 4 hours but at higher temperatures the inactivation increases rapidly

The time course of the inactivation was studied at +37° C With the plasminogen concentration and conditions used in the experiment the inactivation followed approximately a first-order reaction with respect to time However, there was a deviation from the first order during the later part of the reaction With increasing time, the inactiva

tion slowed up, possibly because the denaturated plasmin protected the residual plasmin

The over-all reaction of activation and inactivation

If plasminogen is activated with urokinase at $+37°$ C, the formed plasmin will simultaneously be inactivated If the plasminogen concentrations are not too high the activation will follow a first-order reaction with respect to time as pointed out before The time course of the inactivation of the formed plasmin will also approximately follow a first order Thus, the rate equation of the over-all reaction will be a differential equation equal to the sum of these two rates and the plasmin amount at the time t can be found by integrating the rate equations according to the method of Lagrange (Sjostrand 1961)

$$B = \frac{k_1U}{k_2 - k_1U} P_m (e^{-k_1Ut} - e^{-k_2t})$$

where B is the plasmin amount at the time t U is the urokinase amount and P_m is the maximal plasmin activity obtained by performing the activation at $+4°$ C k_1 is the rate constant of the activation and k_2 the rate constant of the inactivation

An experiment which showed that the data obtained nearly followed a theoretical curve calculated according to the equation was performed

The inactivation was minimal during the initial part of the over-all reaction Thus, a one stage method is preferable for the determination of urokinase activity

VII Theoretical basis and standardization of the one-stage lysis time method for determination of urokinase

Various methods have been used to determine urokinase Celander and Guest (1955, 1960) and Kjeldgaard and Ploug (1957 a) used the one-stage lysis time method The fibrin plate method according to Astrup and Mullertz (1952) has also been widely used A casein method has been used by von Kaulla (1963) to determine urokinase in urine

The theoretical development and practical performance of a quantitative determination of urokinase in a purified system by means of the lysis time method are described in Paper VII

Theory and development of a formula for a one-stage assay

The method is based on the studies of urokinase activation of plasminogen described in Paper VI As pointed out in this work, the urokinase activation of plasminogen follows a first-order reaction if the plasminogen concentration is not too high, otherwise the reaction follows a zero order

According to the findings reported in Paper VI it is desirable to use the initial part of the reaction during which the inactivation of the formed plasmin is mi-

nimal. Therefore, it was decided to use a one stage lysis time method in which the activation of plasminogen proceeds within the clot. It was also presumed that the activation, at least during the initial part of the reaction when the inactivation is negligible, follows the first-order equation

$$\frac{dP}{dt} = k_1U\,(P_m - P) \qquad (VII\ 1)$$

where P is the formed plasmin and P_m is the maximal activity obtained with the urokinase concentration U which is assumed to be constant during the reaction. k_1 is the rate constant and t is the reaction time. Integration gives

$$-\ln\left(1 - \frac{P}{P_m}\right) = k_1Ut \qquad (VII\ 2)$$

$-\ln\left(1 - \frac{P}{P_m}\right)$ can be expressed as a series

$$\frac{P}{P_m} + \frac{1}{2}\left(\frac{P}{P_m}\right)^2 + \frac{1}{3}\left(\frac{P}{P_m}\right)^3 \cdots\cdots$$

If $\frac{P}{P_m}$ is small the series can be approximated to the first term, which substituted in the equation above gives

$$\frac{P}{P_m} = k_1Ut \qquad (VII\ 4)$$

If the lysis time

$t = T$

and the expression for P (Paper IV)

$$P = \frac{F}{k_3T} \qquad (IV\ 8)$$

in which F is the fibrinogen concentration and k_3 the rate constant, are substituted in equation (VII 4) and the logarithmic form is used, the following expression is obtained

$$\log T = -\frac{1}{2}\log U - \frac{1}{2}\log P_m +$$

$$+ \frac{1}{2}\log\frac{F}{k\,k_3} \qquad (VII\ 7)$$

Because equation (VII 1) was developed on the assumption that the urokinase concentration was constant, the above expression is a solution of equation (VII 2). Consequently, all the terms on the right side are constants and the expression tells us that with these urokinase, plasminogen, and fibrinogen concentrations the lysis time is T.

Experimental verification of the formula

The possibility to use the expression above as a formula for a one-stage assay of urokinase has been investigated experimentally. The range of urokinase, plasminogen, and fibrinogen concentrations where the relationship expressed by formula (VII 7) is valid has also been found experimentally.

It has been shown in Paper VI that there is a proportionality between the urokinase concentration and the initial velocity of plasmin formation. If the formed plasmin (P) is small compared with the maximal activity (P_m), the reaction takes place within the initial velocity range and the relationship between lysis time (T) and urokinase (U), according to formula (VII 7) is valid. However, if the urokinase (U) is varied

the maximal plasmin activity (P_m) will also vary even with constant plasminogen because the maximal activity (P_m) depends on the urokinase amount as well as on the plasminogen amount.

The expression (VII.7) can be used as an equation if a straight line with the slope —0.5 is obtained when log of the lysis time plotted against log of the urokinase concentration at constant plasminogen and fibrinogen. This has been verified experimentally. Thus, the expression (VII.7) can be used as a formula for the urokinase assay within the range of the urokinase concentration used.

Astrup and Egeblad (1965) and Egeblad (1966) found a characteristic difference between the clot lysis produced by preformed plasmin and that produced by plasminogen activators in a one-stage assay. They also found a linear relationship if log of the lysis time was plotted against log of the urokinase concentration. The slope of the line was about —0.5. Their lysis time was read from a thrombelastogram. Celander and Guest (1960) used a one-stage method for the determination of urokinase. A slope of —0.6 is obtained from their figure.

The influence of various plasminogen concentrations

It has to be emphasized that the slope —0.5 is an approximation which will be valid only within a certain range of urokinase and plasminogen concentrations because the series transformation of the equation (VII.2) was made on the presumption that the ratio plasmin/maximal plasmin activity (P/P_m) was small. Therefore, it is necessary to use a high plasminogen concentration, but not so high that the reaction follows a zero order.

According to formula (VII.7), a straight line with the slope —0.5 should also be obtained if the maximal plasmin activity (P_m) is varied. P_m is proportional to the plasminogen concentration if the urokinase amount is constant.

An experiment which verifies the validity of the equation for plasminogen concentrations between 1 CU and 10 CU is presented in the present work. At lower plasminogen concentrations, there will be a deviation from the straight line. This may be caused by inactivation of the formed plasmin because of a long lysis time, or that the quotient $\frac{P}{P_m}$ is so small that the approximation is not valid. Higher plasminogen concentrations than 10 CU have not been investigated.

The influence of the fibrinogen concentration

If the formula (VII.7) is written

$$\log T = -\frac{1}{2}\log U + \frac{1}{2}\log\frac{F}{k\,k_3 P_m}$$

it is evident that the last term denotes the distance of the curve from origin. If the maximal plasmin amount (P_m) is constant, the distance will depend on the fibrinogen concentration (F). With low fibrinogen concentrations the line will be nearer to origin and a shorter lysis

time will be obtained Thus the low fibrinogen concentrations increase the sensitivity of the method This can also be shown by plotting log F against log T A straight line with the slope +0 5 will be obtained This is verified experimentally

The method

If the plasminogen, urokinase, and fibrinogen concentration ranges mentioned above are used, a practical method to determine urokinase in a system with purified components will be obtained

The determination is performed by means of a one-stage assay Equal volumes of plasminogen thrombin, urokinase, and fibrinogen are added in the order mentioned to minimize the fibrinogenolysis The urokinase and the fibrinogen should be added almost at the same time to obtain a real one-stage assay The volume of each component is usually 0 1 or 0 2 ml The temperature is +37° C and the pH is 7 4 The lysis time of the formed clot is recorded and the urokinase concentration is read from a standard curve

The standard curve can be constructed easily as the slope is —0 5 The curve is standardized with urokinase of known concentration A one-stage lysis time assay is performed with a known urokinase concentration The lysis time obtained is plotted against the urokinase concentration on a log x log paper Through this point, a straight line with the slope —0 5 is drawn

It is not necessary to know the exact plasminogen concentration but concentrations close to 5 CU/ml should be used As previously pointed out a low fibrinogen concentration increases the sensibility of the test A concentration of 0 05 % gives high sensitivity With this low fibrinogen concentration, the lysis of the clot can still be observed with good accuracy if a high thrombin concentration is used

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The expression (VII 7) can be used as an equation if a straight line with the slope —0 5 is obtained when log of the lysis time plotted against log of the urokinase concentration at constant plasminogen and fibrinogen This has been verified experimentally Thus, the expression (VII 7) can be used as a formula for the urokinase assay within the range of the urokinase concentration used

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According to formula (VII 7), a straight line with the slope —0 5 should also be obtained if the maximal plasmin activity (P_m) is varied P_m is proportional to the plasminogen concentration if the urokinase amount is constant

An experiment which verifies the validity of the equation for plasminogen concentrations between 1 CU and 10 CU is presented in the present work At lower plasminogen concentrations, there will be a deviation from the straight line This may be caused by inactivation of the formed plasmin because of a long lysis time, or that the quotient $\frac{P}{P_m}$ is so small that the approximation is not valid Higher plasminogen concentrations than 10 CU have not been investigated

The influence of the fibrinogen concentration

If the formula (VII 7) is written

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it is evident that the last term denotes the distance of the curve from origin If the maximal plasmin amount (P_m) is constant the distance will depend on the fibrinogen concentration (F) With low fibrinogen concentrations the line will be nearer to origin and a shorter lysis

mately follows a first-order reaction with respect to time Consequently, the over-all reaction can be approximated to two consequtive first-order reactions

12 that in a one-stage system in which plasminogen is activated into plasmin simultaneously with the formation and lysis of a fibrin clot, there is a rectilinear function in a log x log system between the urokinase concentration and the lysis time of the fibrin clot This function is an approximation applicable only within a certain concentration range The slope of the curve is —0 5

13 that the relationship between the urokinase concentration and the lysis time can be used in a quantitative urokinase assay

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SUPPLEMENTUM 479

STUDIES ON SYNOVIAL FLUID IN ARTHRITIS

I THE TOTAL COMPLEMENT ACTIVITY
II THE OCCURRENCE OF MONONUCLEAR CELLS WITH *IN VITRO* CYTOTOXIC EFFECT

BY

HELGE HEDBERG

LUND 1967

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Contents

This work is in part based on the following publications which will be referred to in the ordinary way

Hedberg H, Nordén Å, Lundquist A and Arzelius B Depression of hemolytic complement activity of synovial fluid in adult rheumatoid arthritis Acta med scand 175 347—351 1964

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General introduction

Complement known to be involved in several immunological reactions has been studied for years especially because of its potential cytotoxic capacity in human diseases where an immunological genesis has been suspected

Most clinical studies on complement and complement components have been made on serum

During the past few years interest has increasingly focused on *in vitro* cytotoxic reactions mediated by lymphoid cells Such reactions were first observed in experimental conditions where lymphoid cells from immunized animals were found to elicit a cytotoxic reaction on tissue cultures of those cells against which the animals had been immunized The *in vitro* technique has made possible studies on various human diseases *e g* multiple sclerosis ulcerative colitis and systemic lupus erythematosus In these studies the lymphoid cells were separated from peripheral blood

In patients with arthritis and effusion there is an opportunity to study a body fluid that has presumably been in close contact with the lesions of the tissues surrounding the joint space This was the main reason why synovial fluid rather than blood was studied in the present investigation

FROM THE LABORATORY OF HAEMATOLOGY DEPARTMENT OF INTERNAL MEDICINE,
AND THE DEPARTMENT OF RHEUMATOLOGY UNIVERSITY HOSPITAL, LUND SWEDEN

I THE TOTAL COMPLEMENT ACTIVITY

Introduction

In various diseases where immune reactions have been suspected to play a pathogenetic role the total C′ activity of serum has been studied (see reviews by OSLER 1961, LACHMANN 1963, MÜLLER-EBERHARD, NILSSON, DALMASSO, POLLEY and CALCOTT 1966, RAPP and BORSOS 1966). Reduced C′ levels of serum have been reported for example in active systemic lupus erythematosus (SLE), acute glomerulonephritis and in some cases of acquired haemolytic anemia.

In rheumatoid arthritis (RA), on the other hand, most investigations have shown the C′ level of serum to be raised (VAUGHAN, BAYLES and FAVOUR 1951 a, SCHUBART, EWALD, SCHROEDER, ROTSCHILD, BHATAWADEKAR and PULLEN 1965 and others), whereas in a few studies the C′ level of serum in RA has been found to be normal (LAURELL and GRUBB 1958) or slightly reduced (PERLICK 1962, GEIDEL, SELLE and SCHABINSKY 1964).

However, it is well established that the C′ activity of synovial fluid is often suppressed in RA, sometimes equally pronounced as in cases of SLE and SLE-like syndromes (PEKIN and ZVAIFLER 1962, HEDBERG, NORDEN, LUNDQUIST and AFZELIUS 1964, PEKIN and ZVAIFLER 1964, BARNETT, BIENENSTOCK and BLOCH 1964, HEDBERG 1963, 1964, FOSTIROPOULOS, AUSTEN and BLOCH 1965, FISH, MICHAEL, GEWURZ and GOOD 1966).

Since our first studies some additional observations have been made and the series comprising RA patients has been extended. In order to obtain a better survey, the results on the whole series of arthropathies studied are presented below. The results on such a fairly large series as the present one of 100 RA patients were in fact accumulated for the purpose of clarifying the relation between synovial fluid C′ activity and rheumatoid factor (RF) titre in RA. In none of the mentioned reports, all of which dealt with series of RA smaller than the present one, was any relation found between the two parameters in RA.

The present study of the total C′ activity of synovial fluid was inspired specifically by the demonstration by LACHMANN, MÜLLER-EBERHARD, KUNKEL and PARONETTO (1962) that one of the C′ components (β_{1C}) and immunoglobulins were concomitantly bound to the renal lesions of SLE. If in RA mechanisms similar to those in SLE were involved, it was presumed that a local fixation of C′ during the passage of serum proteins through the inflamed synovial membrane would manifest itself by a reduction of the C′ activity of synovial fluid.

had back symptoms suggesting a diagnosis of ankylosing spondylitis. Nor were psoriatic changes of the skin and/or nails found in any of the patients accepted as RA in the absence of a positive rheumatoid factor (RF) test. LE cell tests were made in 60 out of the 100 RA patients: 58 turned out negative and 2 doubtful. These tests were made predominantly when SLE or SLE like syndromes could be suspected.

Instead of classifying the RA cases into definite and classical RA as proposed by the above mentioned authors the RA cases were classified as follows:

RA with nodules if the patients had subcutaneous nodules at the time of examination. All patients in this group had shown a positive RF test at least once. Two patients with a concomitant psoriasis (SSC* titres 256 and 1024 respectively) were included in this group. This group totalled 27 patients, all adults, 18 females and 9 males.

'SSC pos' RA if a positive SSC test had been observed at least once. In most patients the SSC test had repeatedly turned out positive. Included in this group were 49 adults, 29 females and 20 males, and 7 with juvenile RA, all girls.

'SSC neg' RA if no positive SSC test had been observed. In many patients belonging to this group the SSC test remained negative when the tests were repeated one to three years after examination. In several of these patients the SSC test turned out negative repeatedly (3 to 7 times). Included in this group were 12 adults, 9 females and 3 males, and 5 juveniles, 2 girls and 3 boys.

The reason for considering RA patients with subcutaneous nodules as a separate group of RA will be mentioned below. Once this group was separated it was necessary to use a classification other than that of 'classical and definite RA'. A distinction between 'SSC pos' and 'SSC neg' RA was made in order to attach importance to the SSC test.

The classification used must to some extent be considered arbitrary for e.g. the following reasons: the varying time between onset of joint symptoms and appearance of a positive RF test (see DIXON 1960), the inconstancy of subcutaneous nodules and the varying frequency with which the SSC tests had been made.

Except some of the patients with early RA for less than a year all RA patients examined showed the usual pattern of X-ray changes compatible with diagnosis of RA. In the group of SSC neg RA 15 of 17 patients showed X-ray changes including erosions, one (incompletely examined) showed destruction of cartilage alone.

The mean age of the adult patients in the three RA groups discerned was similar, being 51 (range 38—67) in RA with nodules, 51 (range 26—69) in SSC pos RA and 52 (range 33—64) in SSC neg RA.

Systemic lupus erythematosus (SLE) and SLE like syndromes

Nine females aged 19—55 (mean 40) were labelled SLE or SLE like syn-

* SSC = sensitized sheep cell (see methods)

Materials and methods

The clinical material

Mode of selection

Almost all patients studied had been hospitalized at least once because of joint disease. The majority of patients with inflammatory arthropathies had been admitted to the Department of Rheumatology, University Hospital of Lund (during the years 1962 to 1966), the single special department for rheumatic diseases in the southern part of Sweden.

With the exception of some of the patients with lesions of the menisci, where synovial fluid was secured at arthrotomy, synovial fluid was aspirated from a joint with signs of effusion, generally the knee joint. In most cases fluid was aspirated as a therapeutic measure in association with subsequent intra-articular injection of corticosteroids.

Fluid was not aspirated from joints injected during the two weeks preceding examination (two exceptions).

Haemolytic samples were not accepted; traces of admixture with erythrocytes were disregarded.

RA patients with effusion of short duration and SLE patients were specifically looked for, as were RA patients with subcutaneous nodules during the later part of the study. Except for the limitations mentioned there was no deliberate selection. Thus fluids from patients with different degrees of activity of the disease and with synovitis of different degrees of intensity were studied.[1] When fluids from several joints were aspirated simultaneously, the patient was represented by fluid from the knee joint or, if fluids from both knee joints were aspirated, by fluid from the right knee joint.

The clinical material totalled 174 patients: 147 had adult and 27 juvenile forms of arthropathies.

Rheumatoid arthritis (RA)

Cases of adult and juvenile arthritis were accepted as RA if they satisfied at least 5 of the criteria proposed by ROPES, BENNETT, COBB, JACOX and JESSAR (1958), and if they showed a symmetrical involvement of at least the small joints of hands and/or feet. None of the patients accepted as RA

[1] Attempts to evaluate the intensity of synovitis were made by means of clinical criteria such as tenderness, joint heat and pains on passive motion. As a "measure" of the activity of the disease as a whole the ESR (WESTERGREN) was used.

with peripheral polyarthritis. All had bilateral X ray changes of the sacro iliac joints, 5 also had X ray changes of the spine: three of these had bony ankylosis of the spine. Two with slight to moderate pains and stiffness of the lower back (no signs of prostato vesiculitis) lacked detectable X ray changes of the spine. Two of the patients (X ray changes of the spine) had signs of active prostato vesiculitis and some had fibrotic changes of the prostate and/or vesicles.

Three juveniles, all with bilateral sacro iliitis and atypical polyarthritis were included as ankylosing spondylitis. Two had squaring of the vertebrae and one pronounced destruction of the bone in the upper cervical spine. The pain and stiffness of the back were slight to moderate. Two were boys with onset of peripheral joint symptoms at 9 and 11 respectively and one was a girl with onset at 12.

Uro polyarthritis

Eight males aged 17—53 (mean 30) fullfilled the criteria proposed by OLHAGEN (1960) for a diagnosis of uro polyarthritis. Five with acute forms had a full Reiter syndrome and 3, all with active prostato vesiculitis, had chronic forms. One of the latter had and two lacked signs of sacro iliitis.

Psoriatic arthropathy

In one girl and 13 adults, 5 females and 8 males, the disease was labelled psoriatic arthropathy. All had negative RF tests in serum and synovial fluid, subcutaneous nodules were not present. Ten patients with skin and nail changes had arthritis involving the distal interphalangeal joints. X ray films of these joints taken on 7 of the patients showed erosions or bony ankylosis.

In four of the (adult) patients, all with sacro iliac arthritis, the distal interphalangeal joints were not involved. In conformity with BAKER, GOLDING and THOMPSON (1963) these patients were accepted as cases of probable psoriatic arthropathy.

All patients except one had typical skin changes. In one of the patients with probable psoriatic arthropathy psoriasis was confined to the nails (cultures for pathogenic fungi negative). The psoriatic nature of the arthritis and the nail changes in this patient was also suggested by a highly abnormal glucose tolerance test (see REEDS, FUSARO and FISHER 1964). Highly abnormal or equivocal glucose tolerance tests were observed in several of the patients with psoriatic arthropathy, including one of the other patients labelled probable psoriatic arthropathy, although so far never in sero positive RA (16 patients). All 14 patients of this group were treated together as one group of psoriatic arthropathy if not otherwise stated.

Other sero negative inflammatory arthropathies

One male aged 34 had a sero negative polyarthritis associated with ulcerative colitis.—One female, aged 25, had a transient arthritis of one knee joint probably most closely related to "post infectious rheumatism" of the type de-

scribed by JONSSON (1960).—One male aged 38 had a gouty arthritis verified by biopsy.—One boy aged 17 had a transient effusion of the knee joint without other signs of arthritis.

One patient with onset of joint symptoms at 15 had an atypical polyarthritis with predominant involvement of the large joints.—One boy with onset of peripheral joint symptoms at 10 had a polyarthritis of non-RA type associated with ulcerative colitis and psoriasis; there was no involvement of the sacro-iliac joints nor of the distal interphalangeal joints.—One boy aged 12 had a septic arthritis of the knee joint (culture of synovial fluid showed growth of group A streptococci).

Oligo-arthritis (juvenile)

Eight juveniles, 4 girls and 4 boys, all with a fairly mild arthritis involving a few joints alone, were accepted as oligo-arthritis essentially according to ANSELL and BYWATERS (1962). These authors considered oligo-arthritis one form of 'probable STILL's disease'.

A few patients originally included were excluded because of doubt as to the diagnosis. One (adult) patient with RA was excluded because of recently made (partial) synovectomy.

Methods

The synovial fluid was allowed to clot for two hours at room temperature and was then centrifuged.

Venous blood simultaneously drawn was treated in the same way.

The samples were preserved at about −30°C.

Determination of the no. of $C'H_{50}$ units per ml was performed according to a micro method described by WASSERMAN and LEVINE (1961).

Diluent. Isotonic Veronal buffer (pH 7.2—7.5) containing 0.0005M $MgCl_2$ and 0.00015M $CaCl_2$ and 0.1 per cent gelatin was used as diluent for all reagents.

Sheep erythrocytes. Equal amounts of sheep blood and modified ALSEVER's solution (ALSEVER and AINSLIE 1941) were mixed and kept aseptic at +2—+4°C for 3—6 days before use.

Before use the sheep erythrocytes were washed until the supernatant was free from microscopically detectable haemolysis. The suspension of erythrocytes was adjusted so as to give an extinction value of .710—.840 at 408—412 mμ when diluted 1:16 (after complete haemolysis with distilled water). Minor variations of the 100 per cent haemolysis extinction were of no importance. In one series of determinations the mean extinction for 100 per cent haemolysis was .800 and the standard deviation .029. In this series no relation was found between the 100 per cent haemolysis extinction and the C' level of a serum sample repeatedly determined.

Sensitized sheep erythrocytes (EA) were prepared by mixing the standardized suspension of erythrocytes with an equal volume of amboceptor diluted 1:40 000. The mixture was incubated at 37°C for about 30 minutes during moderate shaking. One and the same amboceptor was used throughout the study.

MILGROM (1963 a) described an agar gel diffusion technique for distinguishing IgG and IgM haemolysins. Agarose gel plates containing sheep erythrocytes were prepared and wells for C' and amboceptor were made, both reagents being used undiluted. The plates were incubated for 20 hours at 4° C and for one hour at 37° C. By this technique it could be shown that the amboceptor used contained one single detectable sensitizing component. This component yielded a strong and distinct haemolytic zone close to the amboceptor well and far from the C' well, suggesting that the sensitizing component was of IgM rather than of IgG type.

Furthermore, pre-treatment of the amboceptor with 0.2M cystein-HCl (final dilution) at room temperature for 4 hours (pH 7.3, no subsequent dialysis) resulted in almost complete disappearance of the haemolytic zone, indicating that the sensitizing component was a macroglobulin (see DEUTSCH and MORTON 1957). Appropriate control experiments suggested that cystein-HCl did not in itself appreciably inhibit the haemolysis of EA by C'. This procedure was known from previous studies completely to destroy the RF activity (by the latex test) of RA sera, while the anti-streptolysin activity of human IgG was scarcely affected (HEDBERG, unpublished observations).

From the above-mentioned results it was concluded that the sensitizing capacity of the amboceptor used was predominantly due to its content of anti-sheep erythrocyte haemolysins of the IgM type.

The haemolytic C' activity was assayed in a total volume of 0.8 ml which contained 0.1 ml of the standardized suspension of EA (about 6×10^7 EA/ml) and varying volumes of diluent and primary dilutions of sample (constriction pipets) according to the following scheme:

Sample (ml)	0.2	0.3	0.4	0.5	0.6
Diluent (ml)	0.5	0.4	0.3	0.2	0.1
EA (ml)	0.1	0.1	0.1	0.1	0.1

Before the samples were finally diluted, primary dilutions of serum—generally 1:500—and of synovial fluid—generally 1:300—were made. These primary dilutions of the sample were kept in ice water until finally diluted.

The mixture of EA and dilutions of the sample was incubated at 37°C ± 1°C for 60 min during moderate and continuous shaking. After incubation remaining erythrocytes were spun down, the extinction of the clear supernatant was read in 1 cm micro cuvettes (1 ml) at 408—412 mμ in a Beckman DU spectrophotometer. The degree of haemolysis was expressed in per cent of the 100 per cent haemolysis after the latter had been corrected for spontaneous haemolysis.

By use of von Krogh's formula the 50 per cent haemolysis end point was determined graphically as described by MAYER (1961). Only haemolysis percentages between 18 and 82 were accepted. Generally three observations were obtained between 18 and 82 per cent haemolysis. The regression line was drawn by eye; the observations between 18 and 82 per cent haemolysis were not allowed to deviate (vertically) more than 10 per cent from the corresponding value of the ordinate on the regression line. If this happened or if only two points or less between 18 and 82 per cent haemolysis were obtained, the determination was repeated. In order to avoid interaction by heterophile antibodies at low dilutions of synovial fluid with an extremely low number of $C'H_{50}$ units, such fluids were considered to contain 32 $C'H_{50}$ units (see HEDBERG 1963).

Table I. Standard deviation (SD) of the determination of the number of $C'H_{50}$ units in 10 sera used as a control of the haemolytic system. Below, the control sera are listed in chronological order

	Control serum number									
	1	2	3	4	5	6	7	8	9	10
No. of determinations	17	17	22	36	53	22	21	17	21	29
Mean	1133	1541	1405	1086	903	871	1006	1191	1175	1203
SD	72	115	75	129	43	54	70	134	60	21
Coefficient of variation	6.3	7.5	5.3	11.9	4.7	6.3	6.9	11.3	5.0	1.8[2]

[1] During the period when each control serum was used there was no tendency towards a change of titre level with time. Coefficient of variation = SD in per cent of the mean.
[2] One additional dilution interposed between the standard dilutions.

In several instances the haemolytic C' activity was determined on two occasions. The standard error of 22 such double determinations on serum was ±6.5 per cent (of the lowest value).[1] For determinations on synovial fluid the standard error was ±8.3 per cent for 16 double determinations. See also Tables I and III.

Leaving the blood or synovial fluid at room temperature for 5 hours instead of 2 did not influence the haemolytic activity, nor did treatment with hyaluronidase (HYALAS® Leo, *120 viscosity reducing units per ml* for 20 min at 37°C). Inactivation of the sample (56°C, 30 min), treatment with immuno precipitates or zymosan completely abolished the haemolytic activity.

At each determination of the no. of C' units per ml a control serum was included. As shown in Table I the methodological error of these determinations was small. It was also evident from the results concerning the control sera, which covered a period of about 4 years, that the stability of the test system used must be considered good.[2]

The protein content of serum and synovial fluid was determined either by a Kjeldahl method (Miller and Houghton 1945) or by a biuret method (Kingsley, Reinhold, Steward, Gilman and Weichselbaum 1953). The two methods showed good agreement.

The protein pattern of serum and synovial fluid was determined by paper electrophoresis according to the method described by Laurell, Lal

[1] The standard error of a single determination = $\pm\sqrt{\frac{\sum d^2}{2N}}$ where d is the differences observed and N the number of double determinations (Dahlberg 1940).
[2] The present study started in May 1952.

RELL and SKOOG (1956). At this procedure 20 μl of synovial fluid was applied on the paper instead of the ordinary 10 used for serum; synovial fluid was applied after treatment with hyaluronidase as described above.

Rheumatoid factor tests. The sensitized sheep cell (SSC) test was made according to the method of WINBLAD (1952) as modified by THULIN (1955).* The SSC titre was determined in whole serum alone. A titre of 64 was considered positive, a titre of 32 doubtful and a titre lower than 32 clearly negative.

The latex fixation (or agglutination) titre (SINGER and PLOTZ 1956) was determined on the euglobulin fraction of serum and synovial fluid, the latter being pre-treated with hyaluronidase as described above.

Euglobulin was prepared either by the procedure described by ERICKSON, VOLKIN, CRAIG, COOPER and NEURATH (1947) or by dialysis against distilled water for 16—18 hours at 2—4°C (pH 5.5—6.5). Euglobulin fractions prepared according to the two methods yielded comparable titres.

The euglobulin fraction diluted 1:10 to 1:320 was contained in a volume of 0.2 ml using a glycine buffer as diluent (pH 8.2—8.4).

One and the same batch of human gamma globulins (KABI, Stockholm) and one and the same batch of latex particles (Dow Chemical Co., 0.81 micron) were used throughout the study.

The reproducibility of the determination of the latex titre of synovial fluid was essentially comparable to that previously described for the serum titre (HEDBERG 1961). Whereas the latex titres in non-RA arthritides proved reproducible, a tendency could be traced in RA for the synovial fluid titres to be less reliable in the titre range < 10—40 than at higher titres. In 3/4 of the fluids with latex titres below 80, attempts were made to make the titres more reliable by determining the titre twice or sometimes three times. A titre obtained twice was accepted, occasionally, in the case of a two-tube difference, the intermediate titre was accepted as the true one. A latex titre of 20 in serum or synovial fluid was considered positive.

Antinuclear activity of serum was determined according to a method described by JONSSON (1967) by means of indirect fluorescent antibody technique. About 35 per cent of the tests were made in connection with examination, the remainder 1—3 years later. The latter tests showed a distribution over different arthritis groups which was comparable to that of the former.

Statistical methods. Besides conventional "parametric methods", use was made of "non-parametric methods" (SIEGEL 1956).

SPEARMAN'S r_s stands for SPEARMAN'S rank order correlation coefficient (corrected for ties).

When frequencies had to be compared the χ^2 test was used, if possible.

* Routine tests performed at the Department of Clinical Bacteriology, University Hospital, Lund. Head: Professor H. GRUBB.

Routine tests performed at the State Laboratory of Bacteriology, Stockholm.

In this case the values of χ^2 as well as of p were given.

If the χ^2 test could not be used, the exact test for 2 × 2 tables was used. In this case the p values solely were given.

The different significance levels were symbolized as follows:

$+ = 0.01 < p < 0.05$ = probable

$++ = 0.001 < p < 0.01$ = significant

$+++ = p < 0.001$ = highly significant

Selection of a method for assessment of the relative synovial fluid complement (C′) activity

In order to make comparisons between different patients or groups of patients possible it was necessary to use relative values of the synovial fluid C′ activity rather than the number of C units per ml synovial fluid as they stand

In arthropathies labelled osteoarthrosis lesions of the menisci and loose body of the knee joint it was *a priori* assumed that C′ was not involved in these pathological changes

In these arthropathies (in total 17 cases subgroups A—C of Appendix Table I) there seemed to be a connection between the number of C units of serum (C'_S) and the number of C units of synovial fluid (C'_{SF}) the degree of correlation was statistically significant ($r = +0.657$ $p < 0.01$ Fig 1) Some degree of correlation was also obtained between C'_{SF} and the protein content of synovial fluid ($r = +0.478$ $p = 0.05$ Fig 1) whereas the correlation between the latter parameter and C_S was very low ($r = -0.112$)

The partial correlation between C_{SF} and C_S after correction for the influence of the protein content of synovial fluid was statistically highly significant ($r = +0.810$ $p < 0.001$)

These results were compatible with the assumption that in the arthropathies in question the total C′ activity of synovial fluid derived from blood serum and that serum C here passed through the capillaries subsynovial tissues and the synovial membrane without being appreciably changed

In the whole series of arthropathies studied the C_S and the protein content of synovial fluid varied considerably as did the C'_{SF}

If the synovial fluid serum ratio for the number of C′ units (C'_{SF} C'_S) instead of C_{SF} alone was related to the protein content of synovial fluid the degree of correlation was statistically highly significant ($r = +0.731$ $p = 0.001$ Fig 1)

The degree of correlation between the quotient C'_{SF} C'_S and the protein content of synovial fluid ($r = +0.731$) was not statistically different from that for the relation C'_S to C_{SF} ($r = +0.657$) nor from that for the relation C'_{SF} to total protein content of synovial fluid ($r = +0.478$) Yet the relation C_{SF} C'_S to the protein content of synovial fluid was used for the estimation of the relative synovial fluid C activity because this relation took into account each of the three parameters considered to be relevant

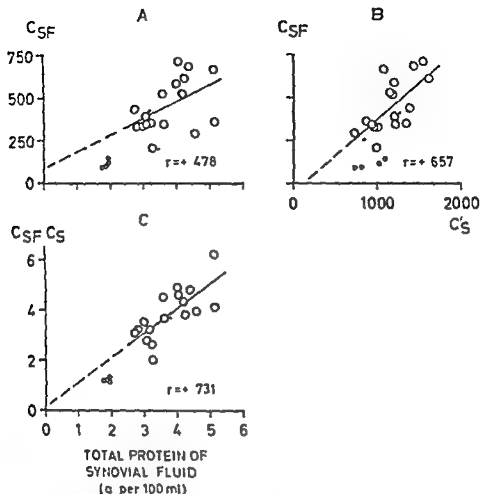


Fig. 1. Relation C_{SF} to concentration of total protein of synovial fluid (A), C_{SF} to C_S (B) and C_{SF}/C_S to concentration of total protein of synovial fluid (C).
C_{SF} = No. of C units per ml synovial fluid
C_S = No. of C units per ml serum
○ = primary observations in 9 cases of osteoarthrosis, 6 of lesions of the menisci, 1 of loose body of the knee joint and 1 of traumatic synovitis. The regression lines and *the r values were based on these 17 primary observations*
∘ = additional observations in osteoarthrosis
• = observations in 4 healthy adults

For the 17 cases considered the relation C'_{SF} C'_S (Y) to the protein content of synovial fluid in g per 100 ml (X) fitted the regression line Y = 0 013 + 0 098X or approximately Y = 0 1X

By making use of this relation the relative synovial fluid C' activity could be estimated For example if the protein content of synovial fluid was 4 00 g per 100 ml the expected value of C'_{SF} C_S (Y) was 0 40 If the observed value of C_{SF} C'_S was 0 30, the relative synovial fluid C' activity was estimated at 0 30 0 40 = 0 75 For the sake of convenience the values of the synovial fluid C' activity thus obtained were multiplied by 100 whereby the relative synovial fluid C activity below simply called synovial fluid C' activity, directly expressed the activity in per cent of the expected

In relation to C' activities estimated in two other ways and especially in relation to the number of C units alone the estimation procedure used yielded a variation of the synovial fluid C' activity values which was fairly low at least within the group of 17 cases considered (Table II)[1]

The synovial fluid C' activity expressed as described fullfilled the following requirements

1) In the whole series of arthropathies studied as well as within various arthritis groups discerned the synovial fluid C' activity varied independently of the variation of the protein content of synovial fluid and also independently of the variation of the C_S at least in non RA seronegative arthritides

Table II Mean and standard deviation (SD) for various estimates of the C activity of synovial fluid 17 cases (9 of osteoarthrosis 6 of lesions of the menisci and 1 of loose body of the knee joint and 1 of traumatic synovitis)

C_{SF} = C H_{50} units per ml synovial fluid

C_S = C H_{50} units per ml serum

TP_{SF} = total protein of synovial fluid (g/100 ml)

Base for the estimation	Mean	SD
Relation C_{SF} C_S to TP_{SF}	1 013	±0 181 (17 9)[1]
Relation C_{SF} to C_S	0 393	±0 101 (25 4)
Relation C_{SF} to TP_{SF}	12 01	±3 33 (27 8)
C_{SF} alone	45 2 2	15 7 7 (34 2)

[1] *SD in per cent of the mean*

2) The synovial fluid C activity could be determined with an acceptable degree of reproducibility (Table III)

It may be added that when in the cases of osteoarthrosis subsequently aspirated synovial fluids were studied these showed a synovial fluid C activity

Retaining the Y values as expressing the C_{SF} C_S ratio measures for X other than the protein content of synovial fluid were also tried for example the globulin content of synovial fluid and the synovial fluid serum ratio for the total globulins as well as that for the α globulins However these other measures for X were found not to be superior to that used i e the protein content

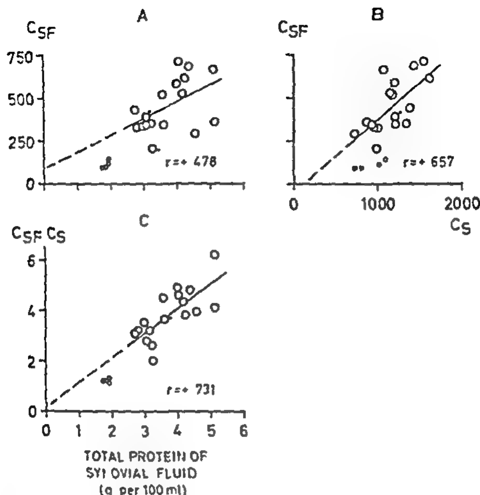


Fig. 1. Relation C_{SF} to concentration of total protein of synovial fluid (A), C_{SF} to C_S (B) and C_{SF} : C_S to concentration of total protein of synovial fluid (C).
C_{SF} = No. of C units per ml synovial fluid
C_S = No. of C units per ml serum
○ = primary observations in 9 cases of osteoarthrosis, 6 of lesions of the menisci, 1 of loose body of the knee joint and 1 of traumatic synovitis. The regression lines and the r values were based on these 17 primary observations.
● = additional observations in osteoarthrosis
• = observations in 4 healthy adults

comparable to that first found (Fig 1)

A few normal synovial fluids were examined for comparison (Fig 1) These showed comparatively low C activities of 67 66 66 and 56 respectively in association with protein concentrations below 2 g per cent[*] However these values were not considered to be suppressed as judged from the confidence limits used (see below)

SUMMARY In order to make a comparison between patients or groups of patients possible a method was devised for the assessment of the (relative) synovial fluid C′ activity This activity could be determined with an acceptable degree of reproducibility

[*] It is doubtful whether or not the estimation procedure really failed at low protein concentrations Possibly these low protein fluids were not strictly comparable to those with a higher protein content with respect to the relative concentration of globulins the latter of which are known to contain the C components so far isolated (MÜLLER EBERHARD NILSSON DALMASSO POLLEY and CALCOTT 1966)

Table III. [illegible] of the [illegible] of the [illegible] (C-act$_{sf}$) in three samples [illegible] different [illegible] determinations

C_{sf} = [illegible] H_{50} units per ml synovial fluid

C_s = [illegible] H_{50} units per ml serum

TP_{sf} = Total protein of synovial fluid [illegible]

$$C\text{-act}_{sf} = \frac{C_{sf}}{C_s} \cdot \frac{10}{TP_{sf}} \cdot 100$$

Determination number	Sample No. 1 C_{sf}	C_s	C_{sf}/C_s	TP_{sf}	C-act$_{sf}$	Sample No. 2 C_{sf}	C_s	C_{sf}/C_s	TP_{sf}	C-act$_{sf}$	Sample No. 3 C_{sf}	C_s	C_{sf}/C_s	TP_{sf}	C-act$_{sf}$
1	[illegible]	17.3	0.43	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4.0	74	[illegible]	1031	0.018	[illegible]	9.4
2	[illegible]	[illegible]	[illegible]	5.0	102	163	1111	[illegible]	3.9	78	[illegible]	[illegible]	0.012	[illegible]	8.2
3	[illegible]	[illegible]	0.47	[illegible]	92	[illegible]	[illegible]	0.16	3.8	42	[illegible]	980	0.016	[illegible]	[illegible]
4	[illegible]	1310	0.43	5.0	84	[illegible]	[illegible]	[illegible]	[illegible]	48	[illegible]	[illegible]	[illegible]	[illegible]	8.8
5	[illegible]	[illegible]	0.43	[illegible]	93	[illegible]	[illegible]	[illegible]	4.0	38	[illegible]	837	0.013	[illegible]	[illegible]
6	[illegible]	1331	0.43	[illegible]	84	178	1133	0.16	3.7	43	[illegible]	[illegible]	[illegible]	5.0	11.6
7	[illegible]	1408	0.43	[illegible]	83	160	[illegible]	[illegible]	3.9	38	[illegible]	[illegible]	[illegible]	[illegible]	10.6
8	[illegible]	1408	0.41	[illegible]	80	156	1290	0.14	4.0	[illegible]	[illegible]	1008	[illegible]	[illegible]	8.7
9	[illegible]	1340	0.38	[illegible]	[illegible]	170	[illegible]	0.14	4.0	[illegible]	[illegible]	976	[illegible]	[illegible]	[illegible]
10	[illegible]	[illegible]	0.41	5.0	82	172	1111	0.16	4.8	42	[illegible]	[illegible]	[illegible]	5.0	11.0
Mean	[illegible]	13.3	0.43	[illegible]	[illegible]	173	1162	[illegible]	3.9	[illegible]	47	963	0.011	[illegible]	9.7
SD	44	[illegible]	0.04	0.10	7.4	8.7	73	0.007	0.11	2.8	3.2	62	[illegible]	0.08	1.07
SD in per cent of the mean	[illegible]	12 %	8.3 %	1.9 %	8.7 %	[illegible]	8.2 %	[illegible]	2.7 %	7.2 %	6.8 %	[illegible]	10.4 %	1.6 %	11.0 %

Table IV Lower confidence limits for synovial fluid C activity in 23 cases of non RA inflammatory arthropathies (subgroups D (7 cases), E (8 cases) and F (4 cases) of Appendix Table I, 3 cases of juvenile ankylosing spondylitis and one of septic arthritis (Appendix Table V))
SD = standard deviation

Deviation from the mean (93.8)		Synovial fluid C activity values		Definition	
		limits	range		
			>67	non suppressed	
−1 SD	(26.6)	67.2			
			42—66		
−1.96 SD	(52.1)	41.7			
			41—25	suppressed at the 1—5 per cent level	suppressed
−2.58 SD	(68.5)	25.3			
			<25	suppressed at the 1 per cent level	

oligo arthritis, SLE and SLE like syndromes were treated together as one group of miscellaneous arthropathies. As mentioned above, the latter seemed to constitute a homogeneous group with respect to the synovial fluid C activity.

As to the three RA groups discerned, the lowest synovial fluid C activities were found in the group of RA with nodules and the highest in the group labelled SSC neg. RA. The group labelled SSC pos. RA showed intermediate values (Fig. 2).

Statistical analysis[1] (Wilcoxon's T test[1]) showed that the synovial fluid C activity values in the group of SSC pos. RA were highly significantly higher than those in RA with nodules and significantly lower than those in the group of SSC neg. RA (Fig. 3). The outstanding low synovial fluid C activity in RA associated with nodules was the reason why RA with nodules was discerned as a separate group.

The synovial fluid C activity in RA with nodules closely agreed with that found in the group of SLE or SLE like syndromes (Fig. 3), where most patients showed extremely low synovial fluid C activities (Fig. 2). In the latter group the estimated value of the synovial fluid C activity of 45 in one of the patients was probably erroneously high (see Appendix Table VI). This case is disregarded below.

For reasons previously mentioned it was decided to use maximized values of the synovial fluid C activity when the no. of C H_{50} units of synovial fluid was extremely low. Such maximized values appeared predominantly in RA with nodules and in the group of SLE and SLE like syndromes (Appendix Tables II and VI). The highest maximized value amounted to 14. Thus all fluids with a C activity of 14 or less—whether maximized values were concerned or not—were considered to have a C activity of 14 when individual values were considered in the statistical analysis.

[1] (= Mann-Whitney's U test) without correction for ties

The C' activity of synovial fluid in various inflammatory arthropathies

The individual values of the synovial fluid C' activity are given in Appendix Tables I—VII together with some other data.

Within the respective arthritis groups concerned juvenile and adult forms showed comparable synovial fluid C' activities (see Fig. 2). For this reason no distinction was made between juveniles and adults. Females and males belonging to the same diagnostic group showed similar C' activities of synovial fluid.

Various non-RA arthritides such as ankylosing spondylitis, uro-polyarthritis as well as some other non-RA arthritides (subgroup I of Appendix Table II) showed on an average very similar synovial fluid C' activities with means of about 100 (Appendix Tables I and VI). They were thus indistinguishable from those found in cases of osteoarthrosis and lesions of the menisci etc. (subgroups A, B and C in Appendix Table II). In the latter case (subgroups A+B+C), however, the variance was probably lower than that in the inflammatory arthropathies mentioned above (variance ratio 2.1; $p = 0.05$).

With respect to the protein content and the white blood cell counts, the synovial fluids in the non-RA inflammatory arthropathies mentioned were comparable to those in RA (see below).

The group consisting of the non-RA inflammatory arthropathies mentioned (totalling 24 cases) served as a reference group with which other arthritis groups could be compared. The synovial fluid C' activity values in the reference group were symmetrically distributed around a mean of about 94. The mean and dispersion in this group also served as a basis for grouping of the synovial fluids and for the definitions (which for the sake of convenience are) used below (Table IV). Owing to the smallness of this group the limits and the definitions are uncertain and merely tentative.

Two other groups of sero-negative inflammatory arthropathies, psoriatic arthropathy and (juvenile) oligoarthritis, both showed on an average synovial fluid C' activities essentially comparable to those found in uro-polyarthritis and ankylosing spondylitis (Fig. 3).

Fig. 2 shows the C' activity of synovial fluid in the whole series of various arthropathies studied. Arthropathies other than RA, psoriatic arthropathy

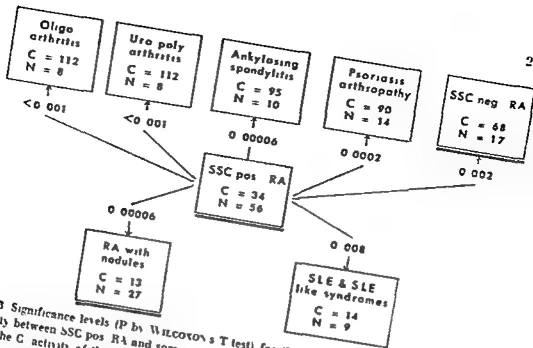


Fig 3 Significance levels (P by Wilcoxon's T test) for the difference in synovial fluid C activity between SSC pos RA and some other arthritides
C = The C activity of the median
N = No of cases

with nodules 32/56 for SSC pos RA and 4/17 for SSC neg RA

SUMMARY Mean C activities of synovial fluid ranging from 80 to 110 —indistinguishable from those found in osteo arthrosis and lesions of the menisci—were found in the following arthritides ankylosing spondylitis uro polyarthritis (Reiter's syndrome) psoriatic arthropathy and (juvenile) oligo arthritis

The group labelled SSC neg RA showed synovial fluid C activities essentially comparable to those found in psoriatic arthropathy and other sero negative arthritides mentioned above

The group labelled SSC pos RA showed significantly lower synovial fluid C activities in relation to SSC neg RA

The lowest synovial fluid C activities observed in RA as a whole highly significantly lower than those found in SSC pos RA appeared in the group labelled RA with nodules Here the synovial fluid C activities were on an average as markedly suppressed as those observed in a small group of SLE or SLE like syndromes

CHAPTER 5

The C' activity of different synovial fluid samples derived from the same patient

In order to study the intra-individual variation of the synovial fluid C' activity, synovial fluids were simultaneously aspirated from different joints of the same patient. In this way a comparison could be made between the C' activity of two fluids derived from each of 23 RA patients (Appendix Table VIII). The results (Table V) showed that the average C' activity of the new series of fluids thus obtained (generally from the left knee joint) was comparable to that of the first series of fluids (generally from the right knee joint). In relation to the between patient variance, which was similar to that for the whole series of RA, the between joint variance was significantly lower (Table V). This corresponded to some degree of correlation between the C' activity of the two fluids simultaneously aspirated (SPEARMAN's $r = +0.46$, $p<0.025$). On the other hand in approximately 2/3 of the patients (Appendix Table VIII) the difference in C' activity between the two fluids exceeded the methodological error expressed as 2 standard deviations (see Table III). In several cases the difference was considerable (Appendix Table VIII).

In several patients synovial fluid was aspirated from different joints (of the same patient) either simultaneously or on different occasions. The results summarized in Table VI showed that in all arthritis groups concerned except SSC pos. RA the distribution of the synovial fluid C' activity of the 2nd joint series was very similar to that of the 1st joint series.

In arthritides other than RA 8 patients showed a non-suppressed C' activity in both synovial fluids, while one with probable psoriatic arthropathy showed a suppressed C' activity in both synovial fluids (Table VI).

As already mentioned new synovial fluids aspirated in cases of osteoarthrosis showed non-suppressed C' activities as high as those of the synovial fluids initially aspirated (Fig. 1).

In RA with nodules as well as in SSC neg. RA it was found that both synovial fluids from each patient showed either a suppressed or a non-suppressed C' activity. In RA with nodules tenosynovitis fluid was studied in three patients: all showed suppressed C' activities with values of 33, 16 and 6 respectively. From these results and the distribution of the synovial fluid C' activities in RA with nodules (Table VI) it was concluded that

Table V Comparison between the C activities of different synovial fluids simultaneously aspirated from different joints of the same RA patient (see Appendix Table VIII) For comparison the series of primary observations in RA is included Fluids with maximized C activity values (C units/ml below 32) are not included

Fluids from	No of cases	C activity of synovial fluid	Mean ± SEM[1]	Analysis of variance of the data of Appendix Table VII: Source	Degree of freedom	Mean square	Variance ratio	P
Different joints	23	1st joint[2]	44 1 ± 5 93	Joints	1	235 13	0 76	>0.2
		2nd joint[3]	39 6 ± 4 61	Patients	22	1009 74	3.272	<0 0
				Error	22	308 63		
				Total	45			
Different patients	92	(1st joint[2])	42.5 ± 3 06	Inter individual variance = 862.3				

[1] Standard error of the mean
[2] *Generally the right knee joint*
[3] Generally the left knee joint

Table VI Distribution of the C activity values of synovial fluids aspirated from different joints of the same patient either simultaneously or on different occasions

Diagnosis	No of observations	Synovial fluid from	C activity of synovial fluid: <25	25—41	42—66	>66
RA with nodules	8	1st joint	4	2	1	1
	[illegible]	2nd joint	5	1	1	1
SSC pos RA	19	1st joint	8	[illegible]	2	[illegible]
	19	2nd joint[1]	3	5	7	4
SSC neg RA	4	1st joint	1	[illegible]	—	1
	4	2nd joint	1	[illegible]	—	1
Psoriatic arthropathy	4	1st joint	1	—	—	3
	4	2nd joint	1	—	—	3
Miscellaneous arthropathies[2]	5	1st joint	—	—	1	4
	5	2nd joint	—	—	2	3

[1] Distribution non significantly different from that of the 1st joint series ($\chi^2 = 4\ 61$ p = 0 10 2 degrees of freedom)

[2] Chronic uro polyarthritis 1 ankylosing spondylitis 2 and oligo arthritis [illegible]

in this group a suppressed C activity was a fairly consistent phenomenon in synovial fluids of various sources

In SSC pos RA on the other hand the 2nd joint series showed a tendency towards a higher synovial fluid C activity in relation to that of the 1st joint series (Table VI) In this group several patients showed a suppressed C activity in synovial fluid from one joint and a high and non suppressed one in fluid from another joint. The results here suggested a random variation

When in SLE and SLE like syndromes new fluids were aspirated these showed suppressed C activities comparable to those initially found. In two of these patients totally seven fluids (five from different joints) showed C activities of 13 23 32 18 20 32 and 8 respectively In a third patient with an SLE like syndrome and a probably erroneously high synovial fluid C activity synovial fluids from three different joints all showed a number of C units that was lower than 32

In addition to a between joint variation a within joint variation was present the latter being studied by aspiration of fluid from the same joint on different occasions. In 22 RA patients' the synovial fluids first aspirated showed a C activity (mean 52 2 standard error of the mean ±8 20) which was on an average similar to that of the subsequently aspirated fluids (mean 47.5 standard error of the mean 5 67 In this series of observations the between patient variance, which was comparable to that for the whole series of RA, was slightly ($p<0.10$) larger than the within joint variance Here too the results suggested a random variation especially in the subgroup labelled SSC pos RA.

When in RA fluids were aspirated on different occasions from the same joint some degree of correlation was found between the C activity of the first fluid and that of the second (SPEARMAN's $r = +0.36$ $p = 0.05$) No relation was found between the length of interval (mean about 14 months) between aspirations of fluid and the difference in C' activity observed The largest differences amounting to about 100 were observed in two RA patients where the interval between aspiration of fluid was half a month and three years respectively In both instances the synovial fluid C activity had decreased from about 130 to about 30 One was a case of RA with nodules which initially showed an exceptionally high synovial fluid C activity (long standing synovitis) The other was a case of SSC pos RA with highly intense synovitis (evident also microscopically) of both knee joints for about 6 months associated with a moderate to pronounced destruction of cartilage. Fluids were aspirated from both joints one from the right knee joint showed a C activity of 10 and the other from the left knee joint a C' activity of 127 After two weeks the C' activity of synovial fluid from the latter joint had decreased to 28 In another case of SSC pos RA a marked decrease from 93 to 21 of the synovial

Patients with maximized values of the synovial fluid C activity not included

fluid C′ activity was also observed during the course of a few weeks. The first fluid was aspirated after a few day's duration of effusion and the second two weeks later.

In 9 non RA patients, [illegible] with psoriatic arthropathy and 3 with ankylosing spondylitis, synovial fluid was aspirated from the same joint on different occasions. All except one of these 18 synovial fluids showed a non suppressed C′ activity. In one patient with probable psoriatic arthropathy the synovial fluid initially aspirated showed a C′ activity of 37 and when aspirated later on an activity of 58.

When marked intra individual differences were found in the case of fluids derived from the same joint or from different joints the differences could not be related to any detectable difference in intensity of synovitis nor to the time for previously given intra articular injections of corticosteroids.

SUMMARY. Synovial fluid aspirated from different joints of the same patient or from the same joint on different occasions were studied. In non RA inflammatory arthropathies such as psoriatic arthropathy, ankylosing spondylitis, uro polyarthritis and oligo arthritis the C′ activity of synovial fluid from different joints was almost consistently non suppressed.

The results suggested than in RA with nodules as well as in SLE and SLE like syndromes the C′ activity of fluids from different joints was more consistently suppressed than in SSC pos RA.

When in RA as a whole additional fluids were aspirated some degree of correlation was found between the C′ activity of the second fluid and that of the first fluid. This was true whether the fluids were aspirated from the same joint or from different joints. In both cases the results suggested a random variation of the synovial fluid C′ activity values.

Presumably no bias was introduced through representing each patient by one single randomly selected synovial fluid.

[illegible]

[illegible]	[illegible]	[illegible]		[illegible]		[illegible]		[illegible]		[illegible]	[illegible]	
[illegible]		9	30 1	1	31 1					1		20 0
[illegible]	SF		(31)	1	(31 1)					6	SF	(1 0)
	S	9	1 17	2	1 0						r S	1 1
RA		0	0 7	1	21	16	1 1	17	17 9	8		0
	1	17	(1 0)	1	(1 1)	1	(1)	1	(16)	1	r	(0 1)
	S	30	1 09	1	1 1	16	1 01	1	1 1	8	SF S	1 1
[illegible]		1	1					6	17			0
[illegible]	S	1	(317)						(1 8)	6	SF	(1)
	?SF ?S	1	9					6	1 1	7	SF ?S	1 1
[illegible]					—				10 0			10 0
[illegible]	SF							6	(10 8)	6	SF	(10 9)
	?S				—				1 00	7	?SF S	1 1
[illegible]							1 7	6		9		0 0
[illegible]	SF						(17)		(0 1)	7	?SF	(1)
	?SF S			—			1 1	6	1 18	9	SF S	1 7
[illegible]		—		—			1 3	11	1 0	13		1 1 6
[illegible]	SF			—			(1 1)	11	(1 0)	13	?SF	(1 1 6)
	?SF S	—		—			1 0	11	0 98	13	SF S	0 99

[illegible]

[illegible]

[illegible]

about 1, as shown in Table VIII essentially similar values were found at different synovial fluid C activity levels. This result suggested that in most cases the bulk of gamma globulins in synovial fluid derived from blood plasma. Obviously a local production of minute amounts of gamma globulins would not be detected by the ordinary paper electrophoretic method used. The possibility that different proteins of synovial fluid may have different elimination rates could also be mentioned as a cause of the sometimes high ratio between the gamma globulins of synovial fluid and those of serum.

White blood cell counts (total leucocytes per mm³ synovial fluid)

In addition to cases of osteoarthrosis where the lowest cell counts were observed (mostly below 1000) synovial fluid cell counts were determined in 91 essentially unselected cases (Table IX).

The overall results showed that, neither in RA as a whole nor in the other arthritides studied, was there any relation between the C activity and the cell counts of synovial fluid (Table IX).

The three RA groups discerned which differed from each other in synovial fluid C' activity showed on an average very similar synovial fluid cell counts amounting to about 16 000 (Table IX).

Within the group labelled SSC pos RA a (non significant) tendency could be traced towards higher cell counts when the C activity decreased, whereas in the group of SSC neg RA the few fluids with C activities below 42 showed fairly low cell counts comparable to those found in SLE and SLE like syndromes (Table IX). Clinical as well as laboratory data (negative ANF and LE cell tests) however argued against these few cases of SSC neg RA being SLE or SLE like rather than RA.

Most patients studied, especially those with RA, had had long standing effusion, often for years. It could indeed be argued whether the effusion in these cases might not often be secondary osteoarthrotic rather than rheumatoid. Although a contribution of secondary osteoarthrotic mechanisms to the effusion could not be excluded, the evidence was against a pure secondary osteoarthrosis being of major importance. In the whole series of 56 RA patients where cell counts were made, counts approaching those found in osteoarthrosis, i.e. below 3000, were found only in two patients, one with a synovial fluid C activity of 39 and one with an activity of 11. In addition, cell counts in RA in the range of 4000 to 6000 seemed to appear as often in early as in long standing effusion. Rheumatoid fluids with a protein content comparable to that of osteoarthrotic fluids showed in relation to these higher cell counts (generally above 5000).

Anticomplementary activity of synovial fluid

In order to study the presence of anticomplementary activity of synovial fluid, especially of fluids with ex

Table IX White blood cell counts of synovial fluid at different C activity levels
Figures in parentheses show no. of cases

Diagnosis	No of cases	White blood cell counts × 10³ per mm³					
		C activity of synovial fluid				Mean ± SEM	Range
		<[illegible]	[illegible]—[illegible]	[illegible]—6[illegible]	>6[illegible]		
RA with nodules	19	1[illegible] (1[illegible])	[illegible]1 9 (3)	[illegible] (1)	1[illegible]0 (1)	1[illegible] ± 2[illegible]	2[illegible]—[illegible]7
SSC pos RA	[illegible]	[illegible] ± 6 9 (8)	13 8 ± [illegible] (8)	1[illegible] 9 (6) / 11 [illegible] ± [illegible] (11)	8 4 (3)	1[illegible] 8 ± [illegible]	[illegible]0—60.5
SSC neg RA	10	6 4 ( )	7 4 (1)	[illegible] (1)	10 (6)	1[illegible] 0 ± [illegible]	3 4—60 9
Total RA	[illegible]	1[illegible] ([illegible]	[illegible] 0 (1[illegible])	11 [illegible] (10)	1[illegible] 7 (10)	1[illegible] 8 ± 1.8	[illegible]—6[illegible]
SLE and SLE like syndromes	6	[illegible]	11 3 [illegible]		—	6 1 ± 1[illegible]	1[illegible]—11 3
Psoriatic arthropathy	8	3 [illegible] (1	1[illegible] ( )		1[illegible] 0 ([illegible])	18 1 ± 6 4	[illegible]—[illegible]0.5
Oligoarthritis	[illegible]				[illegible] 8 ([illegible])	[illegible] 8 ± 10 0	4 [illegible]—[illegible]1 9
Miscellaneous arthropathies	14			11 0 (1)	14 9 (11)	14 1 ± [illegible] 8	3 8—40 0
Postinfectious rheumatism	1				[illegible]10 0	—	—
Septic arthritis	1				390 0	—	—

Standard error of the mean
Mean WBC non significantly higher than that for fluids with higher C activities
Various non RA seronegative arthritides (predominantly ankylosing spondylitis (7) and uropolyarthritis 3)

tremely low C activities a simple non kinetic procedure was used

Fresh as well as heat inactivated fluids (56 °C 30 min) were added to an equal volume of normal serum or synovial fluid the mixture was appropriately diluted and the haemolytic activity of the mixture found was compared with the expected one (see Table X) The degree of anticomplementary activity was defined by the deviation of the activity found from the expected one and expressed in per cent of the latter (see Table X) Most fluids studied by this technique derived from patients with RA SLE and SLE like syndromes fluids with the lowest C activity were studied in a lower final dilution than those with higher C activities

As regards anticomplementary activity no difference was found be

Table X The anticomplementary activity of synovial fluids with different C' activities The fresh fluid to be studied containing n_1 C H_{50} units/ml was mixed with an equal volume of normal serum containing n_2 C H_{50} units/ml The no of C H_{50} units found (n_3) was compared with (n_1+n_2) the expected no of C'H_{50} units the deviation given below was expressed in per cent as follows

$(n_1+n_2-n_3)\times\frac{100}{n_1+n_2}$ For heat inactivated fluids (56°C 30 min) where $n_1=0$ the deviation was similarly expressed
Figures in parenthesis = No of heat inactivated fluids

	C activity of synovial fluid			
	<25	25—41	42—66	>66
No of fluids	20 (7)	10 (4)	6 (3)	8 (3)
Average deviation in per cent	+ 2 71 ± 11 9*	− 1 44 ± 10 3	+ 1 1 ± 11 8	+ 7 4 ± 13 0

* SD

tween fresh and heat inactivated fluids the latter being included in order to study the synovial fluid in as native a state as possible

As shown in Table X where fresh and heat inactivated fluids were treated together no anticomplementary activity was found by the technique used whether the C' activity was suppressed or not six of the fluids with C activities below 25 contained less than 32 C H_{50} units per ml

The results also showed that there was no relation between the RF titre of synovial fluid and the small deviations observed the latter indeed being consistently within the error of the method

The erythrocyte sedimentation rate (ESR)

In SLE and SLE like syndromes low synovial fluid C' activities were generally associated with fairly high ESR values (mean 77) whereas in oligo arthritis non suppressed synovial fluid C' activities were associated with relatively low ESR values (mean 18) In the remaining arthritides studied however the means of the ESR were fairly uniform (44—61) irrespectively of the differences in synovial fluid C activity (Table XI) In addition within the various arthropathies discerned the mean ESR was essentially similar at different synovial fluid C activity levels (Table XI) This was true whether corticosteroids *per os* or ACTH were given or not (Table XI)

Neither in steroid treated nor in untreated RA was there any correlation between the ESR and the synovial fluid C' activity (Table XXI) Thus if the ESR is accepted as a parameter reflecting the disease activity, no relation was found between the latter and

Table XI Erythrocyte sedimentation rate (ESR) at different synovial fluid C activity levels
Figures in parenthesis = No. of cases

Diagnosis	Corticosteroids per os or ACTH	C activity of synovial fluid <25	25—41	42—66	>66	No. of cases	ESR Mean ± SEM[1]	Range
SLE and SLE like syndromes	−	69 (5)	120 (1)	—	—	6	78 —	21—196
	+	76 (3)	—	—	—	7	70 —	61—10
RA with nodules	−	56 (6)	50 (3)	—	—	9	51 ± 7.3	21-
	+	60 (13)	93 (2)	46 (2)	68 (1)	18	63 ± 5.9	27-
SSC pos. RA	−	57 (10)	71 (11)	51 (13)	52 (8)	42	58 ± 4.0	11—
	+	51 (6)	64 (5)	54 (2)	29 (1)	14	54 ± 6.4	29—
SSC neg. RA	−	68 (2)	49 (5)	35 (4)	46 (1)	12	50 ± 0.8	17—1
	+	—	—	—	31 (5)	5	71 —	16—
Psoriatic arthropathy	−	61 (1)	81 (2)	—	55 (9)	11	60 ± 11.1	13—12
	+	—	—	—	63 (3)	3	63 —	25—11
Oligo arthritis	−	—	—	—	16 (7)	7	16 —	3— 3
	+	—	—	—	35 (1)	1	35 —	—
Miscellaneous arthropathies[2]	−	—	—	33 (1)	57 (19)	23	53 ± 7.5	5—12
	+	—	—	—	51 (2)	2	51 —	41— 61

[1] Standard error of the mean
[2] Various (non RA) arthritides—subgroups D—I of Appendix Table I and corresponding juvenile cases of Table X

the synovial fluid C activity. The same was true of various non RA arthritides.

As concerns the intensity of the synovitis the result was similar: no clear cut relation was found between the intensity of synovitis and the synovial fluid C activity. Fairly high synovial fluid C activities were noted in some patients with very intense synovitis and *vice versa*. The reservation here is that it is difficult to obtain but a rough estimation of the intensity of synovitis.

Antinuclear factors (ANFs) in ser

A test for ANFs (in serum) was ma not only in SLE and SLE like sy dromes and in psoriatic arthropat but also in 68 per cent of the RA p tients.

The tests in RA were made with comparable frequency at differen synovial fluid C activity levels.

In addition to SLE and SLE like syndromes a positive test for ANFs was found in an appreciable propor tion of the RA patients with nodules (Table XII). In this group of RA a

Table XII Frequency of positive antinuclear factor (ANF) test in serum at different synovial fluid C' activity levels

Diagnosis	No of cases	Positive ANF test (serum)	C' activity of synovial fluid			
			<25	25—41	42—66	>66
SLE and SLE like syndromes	9	8/9	8/8	0/1	—	—
RA with nodules	21	12/21[1]	8/14	3/4	0/2	1/1
SSC pos RA	34	10/34	3/11	2/10	4/7	1/6
SSC neg RA	13	1/13	0/2	0/2	1/4	0/5
Total RA	68	23/68	11/27	5/16	5/13	2/12
Psoriatic arthropathy	11	4/11	1/1	0/2	—	3/8

[1] Frequency probably higher than that in SSC neg RA ($\chi^2 = 6.35$, $p < 0.05$)

positive test for ANFs occurred probably more often than in the group of SSC neg RA ($p < 0.05$, Table XII) whereas in SSC pos RA an intermediate frequency was found

Within the three RA groups discerned as well as in RA as a whole the frequency of a positive test for ANFs seemed to vary irregularly in relation to the synovial fluid C' activity levels (Table XII) Four out of 11 psoriasis patients showed a positive test for ANFs in 3 out of these 4 the synovial fluid C' activity was higher than 66 (Table XII)

In [illegible] patients who had or had had keratoconjunctivitis sicca there seemed to be a coincidence of a positive test for ANFs and an extremely suppressed synovial fluid C' activity[1] One had a synovial fluid C' activity of 26, and the others had values ranging from 7 to 13 Three of the patients belonged to the group SLE or SLE like syndromes and 6 were RA patients all but one with nodules

[1] The occurrence of keratoconjunctivitis sicca in the present series was not specifically looked for

SUMMARY Attempts were made to relate the synovial fluid C' activity to various parameters such as the protein content and the concentration of gamma globulins in synovial fluid the white blood cell counts of synovial fluid anticomplementary activity of synovial fluid, the ESR and the presence of antinuclear factors in serum No obvious relation was found between the parameters mentioned and the synovial fluid C' activity nor between the latter and the intensity of synovitis

In addition to SLE and SLE like syndromes an extremely suppressed synovial fluid C' activity seemed to coincide with a positive test for ANFs (in serum) in RA with nodules especially if the patient had or had had keratoconjunctivitis sicca

CHAPTER 5

The C' activity of synovial fluid and the duration of effusion

In most RA patients with an effusion of less than 6 months' duration effusion had lasted either for 2 months or less or for more than 3 1/2 months. Owing to this somewhat uneven distribution it was decided to use a dividing line of 3 months for distinguishing between 'early' and 'long standing' effusion.*

In the largest group of RA, i.e. that of SSC pos. RA, three patients with effusion for 3 1/2 to 6 months showed an average synovial fluid C' activity of 35 which closely agreed with that of 31 for five patients with effusion for 7 to 12 months. In long standing effusion for more than 12 months the average synovial fluid C' activity for 37 patients was very similar, i.e. 37. In the following account all effusions lasting for more than three months will therefore be treated together as long standing.

In the group of miscellaneous non RA arthritides as a whole (subgroups D—F of Appendix Table I and corresponding juvenile cases of Appendix Table V) no clear cut relation was found between the C' activity of synovial fluid and the duration of effusion. The findings in psoriatic arthropathy were essentially similar (Appendix Table VIII). Occasionally, e.g. in ankylosing spondylitis and uro polyarthritis, a tendency could be traced towards a somewhat lower synovial fluid C' activity in instances of long standing effusion as compared with that in early one (Appendix Tables I and V).

In the small group of SLE and SLE like syndromes C' activity of synovial fluid was found to be equally suppressed in early as in long standing effusion (Appendix Table VI). Two of the patients with early effusion had had effusion for only 3 and 5 days respectively. Both showed synovial fluid C' activities below 25.

In the group of RA with nodules only three patients had early effusion, all three showing extremely low C' activities comparable to those found in most instances of long standing effusion (Table XIII). However none of these patients presented a fresh effusion of only a few days duration—effusion had lasted for about 8 to 10 weeks. In two of the three patients

* The term 'duration of effusion' was defined by the interval between the first appearance of effusion and the time of aspiration of fluid. In most cases it seemed to be essentially synonymous with the duration of synovitis.

Table XIII Distribution of the synovial fluid C activity values in early effusion of less than 3 months duration and in long standing effusion of more than 3 months duration Figures in parenthesis = No of cases with effusion of less than a week s duration

Diagnosis	No of cases	Duration of effusion in months	C activity of synovial fluid			
			<25	25—41	42—66	>66
SLE and SLE like syndromes	4	<3	3 (2)	1	—	—
	5	>3	5	—	—	—
RA with nodules	3	<3	3	—	—	—
	24	>3	16	5	2	1
SSC pos RA	11	<3	1	4 (1)	2	4 (2)
	45	>3	15	12	13	5
SSC neg RA	3	<3	—	1 (1)	1	1
	14	>3	2	1	3	8
Total RA	17	<3	4	5 (2)	3	5 (2)
	83	>3	33	18	18	14

with early effusion synovial fluid was aspirated from another joint where effusion had lasted for 2 and 4 weeks respectively Both of these additional synovial fluids showed extremely low C activities of 9 and 7 respectively

As judged from the few observations made in early effusion in the group of SSC neg RA these showed synovial fluid C activities similar to those found in long standing effusion (Table XIII) One patient with a synovial fluid C activity of 36 had a fresh effusion of 2 days duration In this patient effusion of both knee joints had appeared simultaneously (during hospitalization) fluid derived from the other knee joint showed a C activity of 39 In this case slight to moderate capsular swelling had been noted before exudation and the patient had had morning stiffness and pains in both knee joints for one to two months

In the group of SSC pos RA 11 patients presented early and 45 long standing effusion (Table XIII) The average synovial fluid C activity in early effusion of 54 7 ± 7 93* was probably higher than that of 36 6 ± 3 46 in long standing ($t = 2\ 09$ $p < 0\ 05$) This difference in C activity between early and long standing effusion was reflected by a somewhat different distribution of the C activity values only one out of 16 fluids with a C activity below 25 was aspirated during early effusion—in this case 2 to 3 weeks after the appearance of effusion—whereas at C' activities above 66 4 out of 9 fluids belonged to the group of early effusion (Table XIII) Two out

* Standard error of the mean

of the 4 fluids mentioned were aspirated after effusion for 2 days or less only one additional patient showing a synovial fluid C activity of 41 had a similar fresh effusion for only 2 days The other two fluids with a C activity above 66 were aspirated after effusion of 2 to 3 weeks duration The most intense synovitis in early effusion (for 2 days) was associated with a synovial fluid C activity of 97

In four patients with SSC pos RA additional fluids from other joints with early effusion were studied two had effusion for about 2 weeks and two for 8 weeks The results were agreed with those of the primary observations the average C activity of these four fluids was 61 range 41 to 8 as compared with that of about for the 11 primary observations at early effusion

In two patients with SSC pos RA and early effusion the C activity was followed for a period of about 3 months In one—with a 3 weeks effusion—the C activity gradually decreased from 49 via 37 to 2 In the other ... tense synovitis fluids from both knee joints were studied after a lag from 33 and 44 to 98 and 21 respectively the C activity rose ... to non suppressed values of 60 and 83 respectively In two patients with RA and nodules similarly studied the samples aspirated from the joint initially studied during early effusion showed ... which were as low as those initially found

In two patients with sero positive RA and long lasting effusion one with nodules and one without marked increase of the synovial fluid C activity to about 100 was observed

In sero positive RA as a whole some degree of correlation was found between the duration of effusion and the synovial fluid C activity during the first 6 months of exudation Spearman's $r = 0.40$ $p < 0.05$

The lower synovial fluid C activity in RA 17 cases was compared with that in non RA sero negative arthritides 17 cases psoriatic arthropathy and gout arthritis included was not first during the first 3 months of exudation ($p = 0.001$ Wilcoxon T test) A similar tendency was true during the first 10 days of exudation

SUMMARY In RA and the group of SLE and SLE like syndromes fresh effusions of only 5 days duration or less were studied only in 6 patients A significant suppression of the C activity of synovial fluid was observed only in two of these patients One had a typical SLE and the other an SLE like syndrome Of the remaining four patients—RA without nodules—two showed higher or non suppressed C activities and two C activities that were suppressed at the 5 per cent level

In addition to SLE and SLE like syndromes extremely suppressed synovial fluid C activities were consistently found only in the group of RA with nodules during the first three months of exudation However in the latter case synovial fluids were studied only when the exudation had lasted for two weeks or more

In SSC pos RA the largest group studied the C activity was found to be higher during the first three months of exudation than in more long standing effusion

In RA as a whole the evidence was against rather than in favour of a suppression of the total C activity of synovial fluid (probable at the 5 per cent level) being an early phenomenon during the course of exudation On the other hand during the first 3 months of exudation this activity was found to be on an average highly significantly lower in RA than in non RA sero negative arthritides

CHAPTER 6

The C′ level of serum

Since some kind of association was found between the C′ level of serum and the ESR it was necessary briefly to consider this relation first Steroid treated patients (including a few receiving ACTH instead of corticosteroids) and untreated ones will be dealt with separately

Table XIX shows the means of the ESR and the C level of serum in some untreated arthropathies as well as in a group of healthy controls The results which suggested a connection between the ESR and the C′ level of serum indicated that in the group of miscellaneous non RA arthritides the mean C′ level of serum as well as the mean ESR were raised to about the same levels as in RA (Table XIX)

In steroid treated as well as in untreated RA no relation was found between the C level of serum and the SSC or latex titre of serum

Table XX shows the means of the C level of serum and the ESR at (three) different synovial fluid C activity levels

In relation to the few cases of untreated SLE and SLE like syndromes untreated RA with almost equally low synovial fluid C activities seemed to show higher C′ levels of serum and lower ESR values (Table XX)

In steroid treated RA comparable amounts of corticosteroids were given at different synovial fluid C activity levels the daily mean doses (as prednisolone) from lower to higher levels being 4 0 3 6 and 3 7 mg in SLE and SLE like syndromes higher doses of on an average 10 mg were given ACTH—on an average 60 IU/week—instead of corticosteroids was given equally often at the three synovial fluid C′ activity levels discerned

The mean C level in the group of steroid treated RA (1129 ± 41 9[1]) was probably lower than that (1221 ± 25 9) in the group of untreated RA (t = 1 97 p = 0 05) the mean ESR in both groups was 56

In the small group of SLE and SLE like syndromes the C level of serum seemed to be lower in untreated than in steroid treated patients whereas the ESR was comparable with means of 78 and 76 respectively (Table XX) In one of the patients with a typical SLE a higher C level of serum of 910 (ESR 46) was observed during treatment

[1] Standard error of the mean

Table VII The mean C' level of serum (C' H_{50} units/ml) and the mean ESR in various arthropathies For comparison a group of healthy controls was included—No treatment with corticosteroids *per os* or with ACTH

Subject	No of cases	ESR	C' level of serum
		Mean ± SEM[1]	Mean ± SEM[1]
Healthy controls	33	7.8 ± 0.25	991 ± 25.6
Subgroups A—C of Appendix Table I	14	13.3 ± 3.0	1131 ± 62.8 [3]
Oligo arthritis	[illegible]	16.4 ± 5.3	1131 ± 99.3
Miscellaneous non RA arthritides[2]	23	53 ± [illegible]	1261 ± 40.3 [3]
RA	62[4]	56 ± 3.6	1421 ± 35.9

[1] Standard error of the mean
[2] Subgroups D—I of Appendix Table I and corresponding (non RA) juvenile cases of Appendix Table V
[3] Probably higher than the mean for healthy controls (p < 0.05)
Highly significantly higher than the mean for healthy controls (p < 0.001)
[4] One juvenile with verified amyloidosis excluded

with ACTH as compared with that of 546 (ESR 30) without ACTH (or steroids)

In untreated RA synovial fluids with the lowest C' activity (below 25) were found to be associated with a mean C' level of serum (1138 ± 33.7[a]) which was probably lower than that (1261 ± 40.3) of the group of miscellaneous non RA arthritides (t = 2.01 p < 0.05 Table XV)

In steroid treated RA the results were principally similar (Table XV) At higher synovial fluid C' activities i e > 25 the mean C' level of serum in RA essentially agreed with that of the group of sero negative miscellaneous arthropathies whether steroid treated or untreated patients were considered (Table XV)

The correlations between the C' level of serum the ESR and the synovial fluid C' activity are reported in Table XVI

In untreated RA as well as in a group consisting of various (untreated) non RA sero negative arthropathies the only correlation found was a positive one between the C' level of serum and the ESR (Table XVI)

In steroid treated RA on the other hand where no correlation was found between the C' level of serum and the ESR the only correlation found was a

[a] Standard error of the mean

Table XV The mean C′ level of serum (C′H_{50} units/ml) and the mean ESR in RA and in SLE and SLE like syndromes Non RA miscellaneous arthritides included for comparison

Diagnosis	Corticosteroids per os or ACTH	C′ activity of synovial fluid	No of cases	C′ level of serum Mean ± SEM[1]	ESR Mean ± SEM[1]
Miscellaneous non RA arthritides[2]	–	>41	23	1261 ± 46.3	53 ± 7.5
RA	–	>41	28	1212 ± 39.1	48 ± 4.7
RA	–	25—41	[illegible]	1329 ± 54.5	67 ± 7.3
RA	–	<25	18	1138 ± 33.7[4]	58 ± 7.1
SLE and SLE like syndromes	–	25—41	1	1057 —	120 —
SLE and SLE like syndromes	–	<25	5	858 ± 175.3	69 ± 19.7
Miscellaneous non RA arthritides	+	>41	6	1403 ± 102.0[3]	55 ± 12.7
RA	+	>41	11	1319 ± 75.1[5]	42 ± 4.8
RA	+	25—41	7	1248 ± 36.7	72 ± 12.1
RA	+	<25	10	975 ± 48.1	57 ± 5.3
SLE and SLE like syndromes	+	<25	3	1207 —	76 —

[1] Standard error of the mean
[2] Various non RA sero negative arthritides (group 4 of Table XIV)
[3] Significantly higher than 1138 ($p < 0.01$)
[4] Probably lower than the mean of 1261 for miscellaneous arthropathies ($p \sim 0.05$)
[5] Highly significantly higher than 975 ($p < 0.001$)

positive one between the C′ level of serum and the synovial fluid C′ activity (Table XVI)

SUMMARY In untreated RA (no steroids) as well as in a group of non RA sero negative (untreated) arthropathies the C′ level of serum was found to be positively correlated to the ESR although not to the synovial fluid C′ activity

In untreated RA with an extremely suppressed synovial fluid C′ activity (values below 25) the mean C′ level of serum was probably lower than that in non RA sero negative arthritides

In steroid treated RA where no correlation was found between the C′ level of serum and the ESR a positive correlation was observed between the C′ level of serum and the synovial fluid C′ activity

In RA no relation was found between the C′ level of serum and the RF titres of serum

Table XVI. Correlations (SPEARMAN's r) between the C' level of serum (C'_S), erythrocyte sedimentation rate (ESR) and C' activity of synovial fluid (C' act$_{SF}$)

Diagnosis	Relation considered	No. of cases	Steroids or ACTH	SPEARMAN's r
Rheumatoid arthritis	C'_S to ESR	62	–	+0.32
		37	+	+0.07
	C'_S to C' act$_{SF}$	62	–	+0.09
		37	+	+0.[illegible]6 **
	C' act$_{SF}$ to ESR	62	–	–0.10
		37	+	–0.20
Miscellaneous non-RA arthropathies[1]	C'_S to ESR	44	–	+0.40
	C'_S to C' act$_{SF}$	44	–	+0.09
	C' act$_{SF}$ to ESR	44	–	+0.01

[1] Groups 2—4 of Table XIV

CHAPTER 7

The C′ activity of synovial fluid and the rheumatoid factor (RF) tests

Evidence has been presented above that the synovial fluid C′ activity is suppressed almost exclusively in syndromes where a positive RF test is a characteristic feature, i.e. in RA and SLE or SLE-like syndromes. Furthermore, even within the group of RA as a whole relatively low C′ activities were found to be associated with a high frequency of clearly elevated RF titres. At relatively high C′ activities, on the other hand, such titres were observed only occasionally (Table XVII). The association with the RF titre was—as shown in Table XVII—statistically highly significant. This was true of each of the RF titres determined, i.e. the SSC titre of serum and the latex titres of serum and synovial fluid. This relation between the synovial fluid C′ activity and the RF titres will be considered in detail below.

Obviously the low frequency of positive RF tests at relatively high synovial fluid C′ activities (above 66, Table XVII) was due to the fact that here the bulk consisted of cases labelled SSC neg. RA with their low frequency of positive RF tests (0/17 by SSC test and 4/17 by the latex tests, Appendix Tables IX and X). On the other hand it should be noted that among the entire series of 100 patients accepted as RA only 13 (with negative RF tests at the time of examination) had shown a consistently negative SSC_S test. Five of these 13 were juvenile with a relatively early onset of joint symptoms (Appendix Table X), where sero-negativity is the rule. Eight were adult females (≈9 per cent of the whole series of adult RA), of whom one had early and transient, and seven long-standing arthritis. These seven, like four out of five juveniles, all had erosive arthritis satisfying 6 or more of the criteria proposed by ROPES *et al.* (1958).

Because the SSC_S titre seemed to be modified by previous treatment with gold salt the RF titres in gold-treated RA will be considered briefly below.

[1] The following abbreviations were used: SSC_S test for the sensitized sheep cell (SSC) test on whole serum, $latex_S$ test and $latex_{SF}$ test for the latex agglutination tests on the euglobulin fraction of serum and synovial fluid respectively.—Although some kind of covariation similar to that described by WINBLAD (1960) seemed to exist between the three RF titres determined, there was no absolute agreement. In addition, the latex tests were more sensitive than the SSC_S test (see Appendix Tables II–VI). For these reasons each of the three RF titres determined will be considered *separately below*.

Table XVII Distribution of the RF titres at different synovial fluid C activity levels in 100 cases of RA

RF test	RF titre	No of cases	C activity of synovial fluid <25	25—41	42—66	>66	χ^2	P
	<32	33	5	6	7	15		
SSC_s[1]	32—64	26	10	6	9	1	29.91[4]	<0.001
	>64	41	22	11	5	3		
	>64	41/100	22/37	11/23	5/21	3/19		
	<20	22	2	2	3	15		
$Latex_s$[2]	20—40	24	6	10	6	2	27.05[5]	<0.001
	>40	53	28	11	12	2		
	>40	53/99	28/36	11/23	12/21	2/19		
	<20	23	2	2	4	15		
$Latex_{sf}$[3]	20—40	22	6	10	4	2	30.27[5]	<0.001
	>40	52	28	10	12	2		
	>40	52/97	28/36	10/22	12/20	2/19		

[1] SSC_s = SSC titre of whole serum

[2] $Latex_s$ = latex agglutination titre of the euglobulin fraction of serum, the titre not determined in one case

[3] $Latex_{sf}$ = latex agglutination titre of the euglobulin fraction of synovial fluid, the titre not determined in three cases

[4] Six degrees of freedom

[5] Four degrees of freedom, at each titre level fluids with C activities above 41 were amalgamated to one group

The SSCs titre in gold treated and untreated RA patients is reported in Table XVIII. Only cases of RA with nodules (A) and SSC pos RA (B) were considered. Untreated refers to RA patients never treated with gold salts and to those treated more than five years before examination; the SSCs titre in the former patients was highly significantly increased above that in patients treated within three years before examination. The three RF titres determined were all non significantly lower in patients treated more than five years before examination than they were in patients never treated with gold salts.

As shown in Table XVIII, SSCs titres below 32 in group A as well as in group B were found with some preponderance in patients who had received gold therapy during the three years preceding examination. When groups A and B were combined the incidence of such low titres was significantly lower in patients treated

Table XVIII The RF titres of serum (by the SSC_S and $latex_S$ tests) and synovial fluid ($latex_{SF}$ test) in relation to previous treatment with gold salts

Diagnosis	No of cases	Time for previous gold therapy (years)	SSC_S titre				
			⩽16	32	64	128—256	512—1024
A	16	>5[1]	—	2	3	5	[illegible]
RA with nodules	3	3—5	—	—	2	—	1
	8	<3	4	1	—	2	1
B	40	>5[1]	6	6	6	15	7
SSC pos RA	8	3—5	1	1	4	1	1
	8	<3	5	1	—	1	1
	56	>5[1]	6	8	9	20	13
A + B	11	3—5	1	1	6	1	2
	16	<3	9	2	—	3	2
			$Latex_S$ titre				
			⩽10	20	40	80	⩾160
	55	>5[1]	4	2	12	16	21
A + B	11	3—5	1	—	3	4	3
	16	<3	4	1	3	5	3
			$Latex_{SF}$ titre				
			⩽10	20	40	80	⩾160
	56	>5[1]	6	7	6	16	21
	9	3—5	—	—	3	2	4
	15	<3	3	3	2	3	4

[1] ">5" includes patients not previously treated with gold salts as well as two patients (both with elevated RF titres) examined during gold therapy. In this group (">5") there was no significant difference in RF titres between untreated and gold treated patients.

[2] SSC_S titres below 32 highly significantly more frequent than in the ">5" group ($p < 0.001$)

during the past three years than in untreated ones ($p < 0.001$). In some of the patients negative SSC_S titres had been observed before treatment; if these patients were excluded the corresponding difference was significant at the 2 per cent level. Patients treated with gold salts three to five years before examination seemed to show an intermediate distribution of the SSC_S titres (Table XVIII).

The $latex_S$ as well as the $latex_{SF}$ titre showed only a statistically non-signifi-

More intense gold salt therapy and more recent treatment seemed to be associated with the lowest SSC_S titres.

Table XIX Correlation (SPEARMAN's r) between synovial fluid C' activity and RF titre
Figures in parenthesis = No. of cases

RF test	Diagnosis	A No gold therapy[1]: No. of cases	Type of effusion: Early	Long standing	Early and long standing	B Gold therapy[1]: No. of cases	Type of effusion: Early and long standing
	The whole series[2]	174	−0.44 (36)	−0.51 (99)	−0.[illegible]3 (173)	32	−0.07
SSCs	RA as a whole	[illegible]1	−0.46 (16)	−0.5 (55)	−0.[illegible] ([illegible]1)	29	−0.12
	RA w. nodules and SSC pos. RA	56	0.46 (13)	−0.3 (43)	−0.40 (56)	27	−0.09
	The whole series	1[illegible]3	0.[illegible]1 (36)	−0.66 (87)	0.64 (173)	32	−0.16
Latexs	RA as a whole	[illegible]0	0.45 (16)	0.51 (54)	−0.57 ([illegible]0)	29	−0.07
	RA w. nodules and SSC pos. RA	55	0.31 (13)	0.31 (42)	−0.37 ([illegible])	25	−0.[illegible]3
	The whole series	174	−0.45 (36)	0.63 (89)	−0.63 (174)	[illegible]9	−0.[illegible]6
[illegible]	RA as a whole	[illegible]1	−0.19 (16)	−0.45 (55)	−0.41 (71)	[illegible]	−0.40
	[illegible] nodules [illegible] pos. RA	58	0.19 (13)	0.17 (43)	0.18 (56)	21	−0.3

1 [illegible] years preceding examination
2 [illegible] SLE like syndromes, psoriatic arthropathy, oligo-arthritis and various [illegible]ro-negative arthropathies listed in Appendix Tables I and V

[illegible] tendency to be lower than 40 when gold salts had been given during the three years preceding examination (p > 0.10, Table XVIII).

In order to obtain a group of RA patients in whom the RF titres, especially the SSCs titres, could be accepted as being uninfluenced—or only negligibly influenced—by previous gold therapy, a limit of five years rather than three was used, although the three-year limit—not the five-year one—was statistically justified. RA patients who had received gold salts during the five years preceding examination are referred to below as "treated".

The synovial fluid C' activity at different RF titre levels in untreated RA

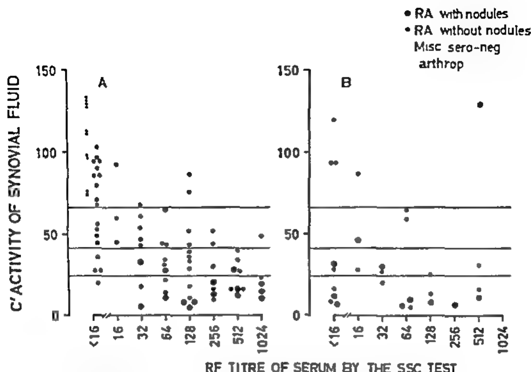
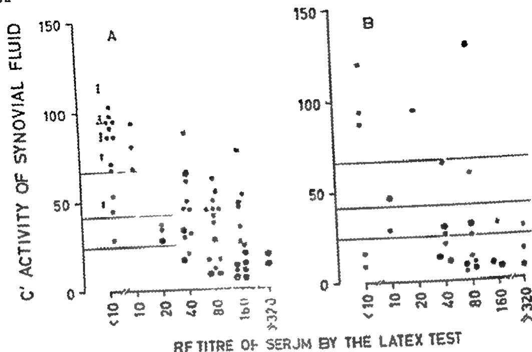


Fig 4 Relation synovial fluid C activity to RF titre of serum by the sensitized sheep cell (SSC) test—100 cases of RA and 25 of miscellaneous sero negative inflammatory arthropathies such as ankylosing spondylitis uro polyarthritis etc (subgroups D E F of Appendix Table I and corresponding juvenile cases of Appendix Table V)
A No gold therapy during the 5 years preceding examination
B Gold therapy during the 5 years preceding examination
The upper line in the Fig is drawn 1 standard deviation below the mean for the group of non RA sero negative miscellaneous arthritides the lowest two lines represent the 5 and 1 per cent limits used (see Table IV)

and in a group of miscellaneous sero negative arthropathies is graphically demonstrated in Figs 4 A—6 A As shown in these figures RA cases with nodules generally occupied the lower range of the synovial fluid C′ activity especially at high titre levels

In untreated RA as well as in the whole series of untreated arthropathies the degree of correlation between the synovial fluid C activity and the RF titre was statistically highly significant (Table XIX) In a diagnostically certain group of RA consisting of RA with nodules and SSC pos RA the results were moreover similar except for the relation synovial fluid C activity to latex$_{SF}$ titre (Table XIX)

Also in the case of the remaining arthropathies studied (arbitrarily treated together in Fig 7) the results fitted in with some kind of association be



Relation synovial fluid C activity to RF titre of serum by the latex test.—The same and symbols as in Fig. 4

tw n the synovial fluid C activity and the RF titre. Most patients with a synovial fluid C' activity below 25—predominantly SLE and SLE like syndromes—showed elevated RF titres by two or three of the RF tests used. At higher synovial fluid C activities however most patients—predominantly oligo arthritis and psoriatic arthropathy—showed consistently negative RF tests.

In conformity with the results in RA suppressed synovial fluid C activities in association with negative or low RF titres were observed in some of the gold treated patients with SLE like syndromes (Figs 4—7). However a similarly low synovial fluid C activity of 18 in association with negative RF tests was found in one patient with a typical SLE not treated with gold salts (fresh effusion for a few days). The arthritis in this case gradually subsided and corticosteroids could be avoided; the RF titres of serum remained negative.

In the whole series of 125 untreated inflammatory arthropathies only 3 showed a suppressed synovial fluid C' activity (below 12) in association with negative RF tests. One was the SLE case just mentioned, one was a juvenile with SSC neg. RA and a synovial fluid C activity of 28 and three were cases of probable psoriatic arthropathy (Appendix Table VII). In two of the latter three cases the latex titre was 10. In the remaining totally 44

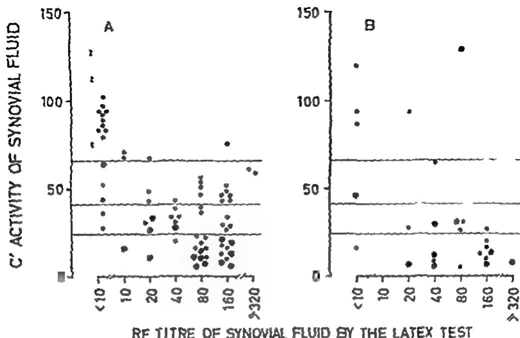


Fig 6 Relation synovial fluid C' activity to RF titre of synovial fluid by the latex test —The same cases and symbols as in Fig 4

cases of inflammatory arthropathies not labelled RA SLE or SLE like syndromes the latex$_{SF}$ titer was < 10 The RF titres of serum in these cases were consistently negative

On the other hand an appreciable proportion of the RA patients with elevated RF titres especially those with elevated latex titres showed a synovial fluid C' activity not considered to be suppressed (Figs 4 A—6 A)

By way of comparison a group of miscellaneous sero negative arthritides was included in Figs 4 A—6 A In untreated RA with negative tests for RFs in serum and synovial fluid the average synovial fluid C' activity of 78 5 ± 5 60 (standard error of the mean) was very similar to that of 92 8 ± 4 04 for miscellaneous sero negative arthritides (47 cases oligo arthritis and psoriatic arthropathy included)

In the present series of RA as well as in several others (see below) the latex test was found to be more sensitive than the SSC test In the whole series of gold treated and untreated RA patients 33 showed a clearly negative SSC$_S$ test (titre below 32) 12 out of these 33 showed an elevated latex$_S$ titre (Table XX) The reverse *i e* an elevated SSC$_S$ titre in association with a negative latex$_S$ (or latex$_{SF}$) titre appeared only occasionally

It is of interest that among the 33 RA patients mentioned an elevated latex$_S$ titre (in association with a clearly negative SSC$_S$ titre) appeared al

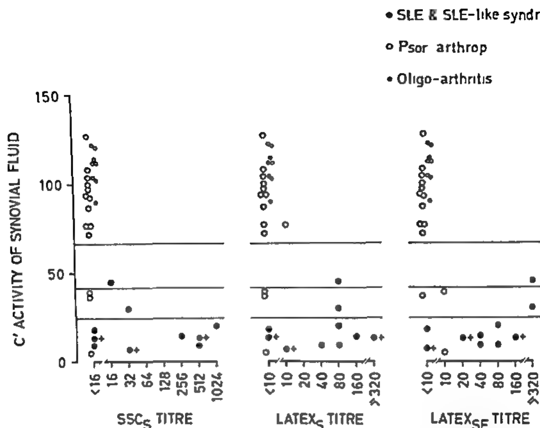


Fig. / Relation synovial fluid C' activity to RF titre in psoriatic arthropathy (13 adults and 1 juvenile), oligo arthritis (8 juveniles) and in SLE and SLE like syndromes (10 adults). In three of the latter, marked with +, gold salts had been given during the 3 years preceding examination.
SSC_S TITRE = RF titre of serum by the sensitized sheep cell test
$LATEX_S$ TITRE = RF titre of serum by the latex test
$LATEX_{SF}$ TITRE = RF titre of synovial fluid by the latex test

most exclusively when the synovial fluid C' activity was lower than 67 and probably more often when this activity was suppressed (values lower than 42) than when it was non suppressed ($p = 0.05$, Table XX). The result was the same if instead of the latexs titre the latexsf was considered. If both these titres were considered only 2 out of 11 RA patients with a suppressed synovial fluid C' activity showed entirely negative tests for RFs in serum and synovial fluid (Table XX). One was a case of juvenile sero negative RA with onset of joint symptoms at nine (Table X) and one was a case of adult gold treated RA. Among the 33 RA patients with a clearly negative SSCs titre the synovial fluid C' activity was somewhat lower in gold treated than in untreated ones ($p = 0.12$ by WILCOXON's T test).

Table XV The latex titre of serum ($Latex_S$) and synovial fluid ($Latex_{SF}$) at different synovial fluid C activity in 33 RA patients with a clearly negative SSC titre of serum (titre below 32)

Previous gold therapy[1]	No of cases	C activity of synovial fluid	$Latex_S$ titre					Frequency of elevated latex titres		
			<10	10	20	40	>40	$Latex_S$ titre >10	$Latex_{SF}$ titre >10	$Latex_S$ and $latex_{SF}$ [illegible]
–	11	>66	9	2	—	—	—	0/11	0/11	0/11
–	6	42—66	2	—	—	2	2	4/6	4/6	4/6
–	3	25—41	1	—	1	1	—	2/3	1/3	2/3
–	1	<25	—	—	—	1	—	1/1	1/1	1/1
+	4	>66	3	—	1	—	—	1/4	1/4	1/4
+	1	42—66	—	1	—	—	—	0/1	0/1	0/1
+	3	25—41	—	1	—	—	2	2/3	2/2	3/3
+	4	<25	2	—	—	1	1	2/4	3/4	3/4
	15	>66	12	2	1	—	—	1/15	1/15	1/15
– and +	7	42—66	2	1	—	2	2	4/7	4/7	4/7
	6	25—41	1	1	1	1	2	4/6	3/5	5/6
	5	<25	2	—	—	2	1	3/5	4/5	4/5
	15	>66	12	2	1	—	—	1/15	1/15	1/15
– and +	18	≤66	5	2	1	5	5	11/19	11/17	13/18
								($p<0.01$)	($p<0.01$)	($p<0.0$
	22	>41	14	3	1	2	2	5/22	5/22	5/22
– and +	11	≤41	3	1	1	3	3	7/11	7/10	9/11
								($p=0.05$)	($p=0.03$)	($p<0.0$

[1] During the 5 years preceding examination

In the case of SLE and SLE like syndromes 4 patients showed a clearly negative SSC_S test in three of these the latex titre was elevated in serum and/or in synovial fluid in one—with a typical SLE—all three tests for RFs were negative In this group all patients except one showed extremely suppressed synovial fluid C activities As said above this patient showed a probably erroneously high C activity of 45 (Appendix Table VI)

In gold treated RA patients (Figs 4 B—6 B) some degree of correlation was found between the synovial fluid C activity and the $latex_{SF}$ titre although not between the former parameter and the SSC_S or $latex_S$ titre (Table XIX)

In the group of RA consisting of RA with nodules and SSC pos RA the lower SSC_S titres in gold treated patients were associated with a somewhat lower rather than a higher synovial fluid C activity ($p \sim 0.50$) in relation to that for untreated ones

As mentioned in ch 5 a distinction was made between early and long standing effusion In the whole series of untreated arthropathies the results in early effusion showed that here as well as in long standing effusion the variation of the synovial fluid C′ activity could be related to a corresponding variation of the RF titres (Table XIX) Lower degrees of correlation between the synovial fluid C activity and the RF titres were obtained (Table XIX) in the smaller series of early effusion in RA as a whole as well as in the group of RA consisting of RA with nodules and SSC pos RA

The relation between synovial fluid C′ activity and RF titres in early effusion of untreated arthropathies is demonstrated separately in Fig 8 Although the degree of correlation was statistically significant (Table XIX) several cases of RA without nodules showed fairly high synovial fluid C activities in association with elevated RF titres of serum In early effusion of RA with nodules extremely low synovial fluid C′ activities were associated with moderately or markedly elevated RF titres of serum (Fig 8) This result in RA with nodules as well as the tendency towards high synovial fluid C activities in proportion to the elevated RF titres of serum in instances of early effusion in RA without nodules were confirmed by the results of additional observations on synovial fluid from a joint other than that primarily studied (Fig 8)

However in sero positive RA the duration of effusion during the first six months of exudation also seemed to be of importance for the degree of suppression of the C activity of synovial fluid (CHAPTER 5) Owing to the limited series of observations it was not possible to decide which of the two parameters was the predominant one

The results in RA seemed to be compatible with the assumption that the C′ activity of synovial fluid during the first time of exudation was to some extent related to the RF titre and to some extent to the duration of effusion

In a few cases studied during the first months of exudation (6 of RA and one of an SLE like syndrome) were additional observations made after varying intervals These observations represented new fluids derived from the joint initially studied In a few instances fluids from another joint were included for the sake of comparison (Fig 9)

In two cases of SSC pos RA, where the interval exceeded 12 months the fluids aspirated later on showed a similar or slightly higher C′ activity as compared with that initially found during the early phase of exudation In these two cases the RF titres either decreased or remained at the same low level (Fig 9) In one of these cases a lower C activity was found in a fluid aspirated (on an earlier occasion) from another joint early during the exudation This lower activity was associated with elevated RF titres (Fig 9)

In one case of an SLE like syndrome where the arthritis ran an intermittent course the interval between

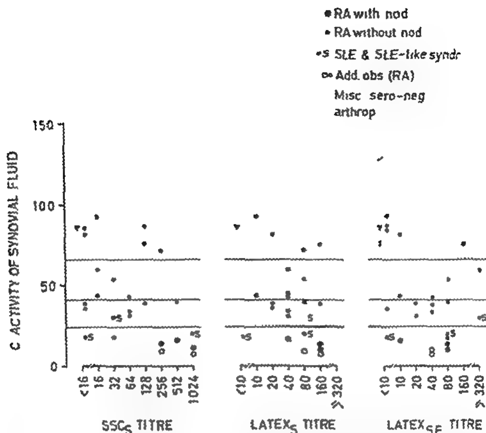


Fig 8 Relation synovial fluid C activity and RF titre in early effusion of less than 3 months duration Thirty six patients (no gold therapy during the 5 years preceding examination) 16 with RA (additional observations on synovial fluid from a joint other than that initially studied are marked by circles) 3 with SLE or SLE like syndromes and 17 with various sero negative non RA arthritides (uro polyarthritis ankylosing spondylitis psoriatic arthropathy oligo arthritis etc)

the two aspirations was almost 3 years In this case a moderate increase of the synovial fluid C activity (from 13 to 32) was found to be associated with unchanged RF titres of serum and with a probable decrease of the RF titre of synovial fluid (Fig 9)

In two cases of RA with nodules early effusion and high RF titres of serum it was found that initially extremely low synovial fluid C activities were followed by equally low activities after one or two months During this time the RF titres of serum remained essentially unchanged at the same high level In one of these cases the latex$_{SF}$ titre increased from 10 to 80 (Fig 9)

In one of two patients with SSC pos RA studied during the early phase of exudation a sudden and transient suppression of the synovial fluid C ac

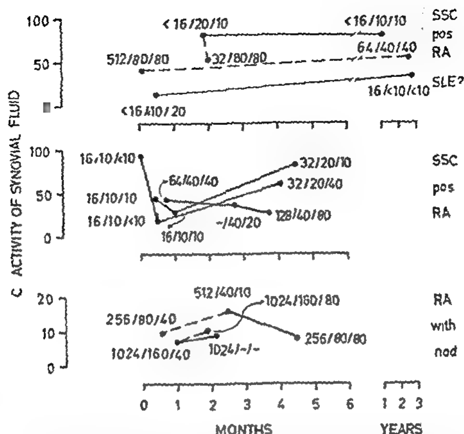


Fig. 9. RF titres and synovial fluid C activity in some cases studied early during exudation as well as later on after varying intervals. The titres by the three RF tests (SSC_S/$latex_S$/$latex_{SF}$) are given at each point symbolizing the C activity. Six cases of RA and one of SLE like syndrome (labelled "SLE?" in the Fig.).
Unbroken lines connect observations on the same joint, broken lines connect observations on different joints of the same patient.
Note the change of the scale in the lower part representing RA with nodules.

tivity was followed after 3 to 4 months by a probable increase of the $latex_{SF}$ titre and a non-significant (one tube) increase of the RF titres of serum. The C activity of synovial fluids aspirated from the other knee joint seemed to behave similarly in these synovial fluids, however, the $latex_{SF}$ titre remained unchanged and negative. In the other patient with SSC pos. RA initially showing a synovial fluid C activity of 42 there seemed to be a slight and gradual decrease of the activity during the subsequent three months. In this case the RF titres of serum and synovial fluid remained essentially unchanged at the same moderately elevated level (Fig. 9).

Because of the observed correlation between the synovial fluid C activity and the RF titres the three RA groups—RA with nodules, SSC pos. RA and

Table XXI Significance levels (P by WILCOXON's T test) for the differences in synovial fluid C activity and RF titres Three groups of RA RA with nodules (16 cases) SSC pos RA (40 cases) and SSC neg RA (15 cases) For comparison the group of psoriatic arthropathy is included (14 cases) Patients who had received gold salts during the 5 years preceding examination were not included

Groups compared	C activity of synovial fluid	SSC_S titre	$Latex_S$ titre	$Latex_{SF}$ titre
RA with nodules and RA without nodules¹	0 000003	0 003	0 01²	0 52²
RA with nodules and SSC pos RA	0 00008	0 08	0 19	0 51³
RA with nodules and SSC neg RA	0 00032	0 000002	0 00003	0 001
RA with nodules and Psoriatic arthropathy	0 00001	0 000003	0 000005	0 00002
SSC pos RA and SSC neg RA	0 0016	0 0000004	0 000004	0 00006
SSC pos RA and Psoriatic arthropathy	0 0003	0 0000007	0 0000001	0 0000006
SSC neg RA and Psoriatic arthropathy	0 16	0 75	0 20	0 48

¹ = SSC pos RA and SSC neg RA
² If gold treated patients were included comparable P values were obtained (P = 0 008 and 0 42 respectively)
³ If gold treated patients were included similar non significant differences were obtained (P = 0 17 and 0 58 respectively)

SSC neg RA which differed significantly in the synovial fluid C activity (Fig 3)—should be expected to differ also in respect of the RF titres As expected from the definition of the three RA groups this was to some extent the case especially as regards the RF titres of serum (only patients not treated with gold salts during the five years preceding examination were considered) (Table XXI)

RA with nodules showed RF titres which differed only non significantly from those found in the group of SSC pos RA (Table XXI) The latter group however was defined by a criterion which is known to be a characteristic feature of the former i e that of a positive SSC_S test Anyhow as judged from the present series RA with nodules could be separated from SSC pos RA more effectively by the synovial fluid C activity than by the RF titres (Table XXI)

On the other hand if a distinction was made between RA with nodules

Table XXII The synovial fluid serum ratio for the latex titre at different synovial fluid C activity levels 74 cases of RA and 6 of SLE and SLE like syndromes *Gold* treated patients included

Figures in parenthesis = cases of early effusion

Diagnosis	C activity of synovial fluid	No of cases	Synovial fluid serum ratio for the latex titre				
			<1/2	1/2	1	2	>2
RA with nodules	>66	1	—	—	1	—	—
	42—66	1	1	—	—	—	—
	25—41	5	—	2	3	—	—
	<25	15 (3)	2 (1)	5 (2)	5	1	2
	Total	22	3	7	9	1	2
RA without nodules[1]	>66	4 (2)	1 (1)	—	2 (1)	1	—
	42—66	16 (5)	1 (1)	3	6 (3)	3	3 (1)
	25—41	16 (3)	3 (1)	2 (1)	6 (1)	[illegible]	3
	<25	16 (1)	—	3 (1)	5	6	2
	Total	52	5	[illegible]	19	12	8
SLE and SLE like syndromes	25—41	1 (1)	—	—	—	—	1 (1)
	<25	5 (2)	1	—	3 (1)	—	1 (1)
	Total	6	1	—	3	—	2
RA SLE and SLE like syndromes	>66	5	1	—	3	1	—
	42—66	17	2	3	6	3	3
	25—41	22	3	4	9	2	4
	<25	36	3	8	13	7	5
	Total	80	9	15	31	13	12

[1] SSC pos RA and SSC neg RA

and RA without nodules (=SSC pos RA and SSC neg RA) the lower synovial fluid C activity in the former group was associated with RF titres of serum (SSCs and latexs titres) significantly higher than those in the group of RA without nodules (Table XXI) there was however still no difference as regards the latexsf titres This means that in (untreated) RA patients with nodules the latex titre of synovial fluid was low in proportion to that of serum

The group of psoriatic arthropathy, included for the sake of comparison in Table XXI agreed most closely with the RA group labelled SSC neg RA with regard to the synovial fluid C activity and the RF titres

When the individual values of the synovial fluid serum ratio for the latex titres was considered in the whole series of RA SLE and SLE like syndromes (previous gold treatment was neglected) this ratio—below called RFsf s ratio—was found to be essen

Table XXIII Means and (inter individual) variances of the synovial fluid C activity values in RA at different SSC titre levels in serum 60 RA patients (gold treated patients and cases of maximized values not included)

	SSC titre of serum			
	≤16[1]	32—64	128—256	512—1024
No of cases	14	15	18	13
Means	77 9	40 1	33 9	23.3
Variances	501 5	284 8	509.2	135 1
Variance ratio[2]	3 71	2 11	3 77	—

[1] Only cases with negative latex titres in serum and synovial fluid
[2] The variance at the highest titre level in the denominator

tially distributed symmetrically around a mean of about 1 (Table XXII un limited values from 2 to 1/2 were not included see Appendix Tables II—VI)

Although the RF_{SFS} ratio must be *subject to a considerable methodologi* cal error it is perhaps worth mention ing that there was a tendency in RA for this ration to be lower than 1 in instances of early effusion ($\chi^2 = 4\ 08$ $p < 0\ 05$ Table XXII) However there was no relation between the RF_{SFS} *ratio* and the synovial fluid C activity whether *early effusion or long* stand ing was considered In both cases the synovial fluid C activity values seemed to be related to the presence of nodules rather than to the RF_{SFS} ratio (Table XXII) This result was *confirmed by the results of additional* observations (see Fig 8)

As shown in Figs 4 A—6 A the aver age synovial fluid C activity in RA seemed gradually to decrease when the RF titre increased With respect to the SSC titre of serum though not to the latex titres of *serum and synovial* fluid the inter individual variation of the synovial fluid C activity tended to be low when the titre was maximal ly elevated (Table XXIII see also Figs 4 A—6 A)

As *mentioned already the synovial* fluid C activity was subject to a some times considerable intra individual variation The degree of this variation similar to that of the inter individual seemed to some extent to be related to the SSC titre of serum For example five patients had SSC titres of serum *of 512 —1024 four out of these (three* with nodules) had synovial fluid C activities of 11—31 and only small between joint differences correspond ing to a between joint variance of 23 3 were found In one of these four pa *tients the C activity of a tenosynovitis* fluid was 6 in the fifth patient excep tionally high C activities of 129 and 89 respectively were noted in synovial fluids from both knee joints At inter mediate (32—256) and clearly nega tive SSC titre levels the inter articular and also the *intra articular* variances

were comparable to the inter-individual ones.

SUMMARY. RA patients treated with gold salts during the years preceding examination showed a lower sensitized sheep cell (SSCs) titre of serum in relation to untreated ones. For this reason a distinction was made between gold treated and untreated patients.

In the whole series of various untreated inflammatory arthropathies studied as well as in untreated RA as a whole a high degree of negative correlation was found between the synovial fluid C' activity and the SSCs titre.

By relating the synovial fluid C' activity to the latex titres of serum and synovial fluid instead of to the SSCs titre the connection between the synovial fluid C' activity and the RF titre was confirmed.

Sero-negative inflammatory arthropathies other than RA, SLE and SLE-like syndromes showed on an average synovial fluid C' activities only non-significantly higher than those found in untreated RA with negative RF tests.

Untreated RA patients with nodules showed highly significantly lower synovial fluid C' activities and significantly higher RF titres of serum than RA patients without nodules.

In cases of a clearly negative SSCs test in gold treated and untreated RA patients the appearance of elevated latex titres in serum and/or in synovial fluid was almost exclusively confined to patients with suppressed synovial fluid C' activity.

CHAPTER 8

Discussion

The present study, like that of PEKIN and ZVAIFLER (1964), yielded evidence for the passage of 'whole' C of serum into the joint space essentially in proportion to the amount of serum protein passing into the joint space.[1] This refers to various sero-negative non-RA arthropathies as well as to normal subjects. In the present study each of the three parameters considered to be of importance was taken into account: the number of C units per ml of synovial fluid (C_{SF}) and serum (C_S) and the total protein content of synovial fluid (TP_{SF}). The C activity of synovial fluid was expressed as $(C_{SF} \times 1000) : (C_S \times TP_{SF})$. The relative C activity of synovial fluid thus obtained yielded a value directly expressing the C activity in per cent of the expected.[2] The C activity of synovial fluid could be determined with an acceptable degree of reproducibility (Table III). By the use of the relative C activity of synovial fluid (below called "C activity of synovial fluid") rather than the number of CH_{50} units per ml synovial fluid as they stand a comparison between patients or groups of patients became possible.

Various mechanisms might be responsible for the suppression of the C activity of synovial fluid, for example inactivation of C by antigen-antibody complexes or by aggregated IgG solely.

BOYDEN, NORTH and FAULKNER stated (1965) that the role played by C in phagocytosis is obscure. FOSTIROPOULOS, AUSTEN and BLOCH (1965) discussed phagocytosis as a possible cause of the suppression of C activity, especially in RA, where synovial fluid leucocytes are known to phagocytose aggregates of RF and IgG (ASTORGA and BOLLET 1964, HOLLANDER, RAWSON, RESTIFO and LUSSIER 1964). However, leucocytes containing such aggregates were found also in some of the cases of psoriatic arthropathy and ankylosing spondylitis, where the synovial fluid C activity is generally non-suppressed. FOSTIROPOULOS, AUSTEN and BLOCH (1965) took the absence of a consistent reduction of synovial fluid C'2 titres in gouty effusions as evidence against phagocytosis as a C-utilizing mechanism of importance for the suppression of the synovial fluid C activity.

The occurrence in *i.a.* rheumatoid

[1] ZVAIFLER and PEKIN (1963) had also shown that in cases of osteo-arthrosis, Reiter's syndrome and gout the synovial fluid/serum ratio for the classical C components equalled that for the total C activity.

[2] The expected value of $C_{SF} : C_S = TP_{SF} \times 1/10$.

synovial fluid of a variety of essentially lysosomal enzymes is presumably in part related to phagocytosis (HAMERMAN, SANDSON and SCHUBERT 1963 and others, see review by JESSAR 1966). Some of these enzymes are proteolytic and might in some way have a bearing on the suppression of the synovial fluid C′ activity. However, rheumatoid synovial fluids have been reported to contain on an average less pepsin and cathepsin than do fluids from patients with osteoarthrosis and traumatic hydrarthrosis. Moreover the trypsin activity was comparable in the three types of fluids studied (VAINIO 1960).

Another proteolytic enzyme, plasmin, has recently been suggested capable of inactivating C′ by simple proteolytic cleavage of C′ components (LAURELL, LUNDH and MALMQUIST 1965) rather than by converting C′1 to C′1 esterase (which is known to destroy C′2 and C′4) as previously supposed. However, in synovial fluid with a suppressed C′ activity neither plasmin activity nor plasmin inhibitors could be detected (HEDBERG 1964).

The C′ activity of synovial fluid in RA as well as in the other arthritides studied was found to vary independently of its white blood cell counts and the concentration of gamma globulins. This agrees essentially with the results of PEKIN and ZVAIFLER (1964) and FOSTIROPOULOS, AUSTEN and BLOCH (1965). Noteworthy are the relatively low white blood cell counts in the group of SLE and SLE-like syndromes in which most synovial fluids showed extremely suppressed C′ activities. The tendency towards low white blood cell counts in SLE as well as the average cell count in rheumatoid synovial fluid found in the present study, agree with previous reports (e.g. JOHANSEN and SYLVEST 1961, HOLLANDER, JESSAR and McCARTY 1961).

In the present study as well as in the studies by PEKIN and ZVAIFLER (1964) and by FOSTIROPOULOS, AUSTEN and BLOCH (1965) it was found that incubation of rheumatoid synovial fluids (with a suppressed C′ activity) with serum or synovial fluid showing a non-suppressed C′ activity did not result in a decrease of the latter. Similar results were obtained with non-RA fluids, all of which had a non-suppressed C′ activity. In these experiments the concentration of synovial fluids with a suppressed C′ activity was higher than that of fluids with a non-suppressed activity although lower than at the determination of the number of $C'H_{50}$ units.

Kinetic studies similar to those made on RA sera by HEIMER, LEVIN and KAHN (1963) were made on heat-inactivated (56°C for 30 minutes) synovial fluid diluted 1:10 (final dilution).* The results showed that 9 non-

* The synovial fluid was pre-incubated with 4 $C'H_{50}$ units (diluted normal serum) at 37°C. Samples were drawn from this mixture every 10 minute during 60 minutes, whereafter the haemolytic activity was determined after an additional incubation time of 60 minutes at 37°C. In these kinetic studies where the haemolytic C′ activity was determined in a larger volume than that used in the routine determinations each C′ unit roughly corresponded to 10 units in the routine determinations.

RA synovial fluids predominantly derived from patients of ankylosing spondylitis and uro polyarthritis, did not inhibit the haemolysis of EA by 4 C units Ten out of twelve rheumatoid fluids significantly inhibited haemolysis 4 out of these 10 fluids inhibited about 2—2 1/2 C′ units and 6 inhibited about 3 units The two fluids that lacked detectable inhibitory capacity had both a suppressed C′ activity with values of 21 and 29 respectively as had 8 of the 10 rheumatoid fluids (C′ activities below 25) that inhibited haemolysis One of the latter had a C activity of 94 there was no macroscopically detectable agglutination of EA (HEDBERG and NORDEN unpublished observations)

The results of the mentioned kinetic studies on rheumatoid synovial fluid did not support a close connection between the C′ inhibiting capacity and the C activity of synovial fluid

In most instances of a suppressed *synovial fluid C activity the number* C H_{50} units was determined in synovial fluid considerably more diluted (1 50 or more see Appendix Tables II—VI) than those subjected to kinetic studies (1 10) In the former case—at dilutions of 1 50 or more—the anticomplementary activity was probably lower than that in the latter case (dilution 1 10) On the other hand the possibility cannot be excluded that some anticomplementary activity was lost by heat treatment of synovial fluid at 56°C for 30 minutes This reservation is based on the observation by FROMMHAGEN and FUDENBERG (1962) that heat treatment of serum at an only slightly higher temperature of 60°C can reduce its anticomplementary activity It is difficult to decide whether the mentioned *in vitro* findings might have any bearing on an *in vivo* depression of the synovial fluid C′ activity

A gamma macroglobulin that inhibited C′ fixation was isolated from RA sera by HEIMER LEVIN PRIMACK CORCOS and NOSENZO (1962) This inhibitor as well as heat inactivated whole serum from RA patients also inhibited haemolysis of EA by C′ the C′ inhibiting capacity of RA serum remained unchanged after absorption of RF (HEIMER LEVIN and KAHN 1963) Even if it has not been shown it seems possible that the C′ inhibiting capacity of rheumatoid synovial fluid is analogous to that of RA serum with its generally raised C level Another possibility is that rheumatoid synovial fluid can contain traces of aggregated IgG

In some rheumatoid synovial fluids *suppressed C activities were found to* be associated with negative or only weakly positive RF titres by the latex test On the other hand a considerable proportion of the rheumatoid synovial fluids with markedly elevated latex titres showed non suppressed C′ activities comparable to those sometimes found in the group of non RA arthritides (Fig 6) In the mentioned kinetic studies on rheumatoid synovial fluid no relation was found between the C inhibiting capacity and the RF titre

In the present study no relation was found between the C′ level of serum and the RF titre The same is true of several other studies (LAURELL

and GRUBB 1958, SCHUBART, EWALD, SCHROEDER ROTSCHILD BHATAVADEKAR and PULLEY 1965 and others), where the C′ level of serum was determined in samples considerably less diluted than in the present one The data mentioned argue against the possibility that RF had interfered at the determination of the number of $C'H_{50}$ units in synovial fluid RF is known to interact with antibodies of the IgG type whereas in determinations of the haemolytic C′ activity C′ interacts presumably predominantly with antibodies of the IgM type coating the surface of the sheep erythrocytes (ZVAIFLER and BLOCH 1962 ULSTRUP 1962 SCHMID and ROITT 1965 and others) By use of a technique described by MILGROM (1963 a) it could be shown that the amboceptor used in the present study contained one single detectable sensitizing component This component diffused slowly (in agaros) and was therefore assumed to represent IgM rather than IgG antibodies

ZVAIFLER (1965) recently suggested that the suppression of the C′ activity in rheumatoid synovial fluids might reflect a local antigen antibody reaction possibly involving antinuclear factors (ANFs) In the present study the lowest synovial fluid C′ activities were found in a group of SLE and SLE like syndromes and in RA patients with nodules In addition to the group of SLE and SLE like syndromes a positive test for ANFs in serum was found predominantly in RA patients with nodules A similar observation has been made by BARNETT LEDDY CONDEMI and VAUGHAN (1965) Nevertheless, neither in the group of RA with nodules nor in the two other RA groups discerned or in psoriatic arthropathy was any connection found between the synovial fluid C′ activity and the presence of ANF activity in serum (Table XII) Furthermore in a limited series of patients, detectable ANF activity in synovial fluid was found exclusively in those with a positive test for ANFs in serum (HEDBERG 1964) Similar results have been published by BARNETT BIENENSTOCK and BLOCH (1964) These authors suggested that the failure to detect ANF activity in all rheumatoid synovial fluids might be attributable either to a failure of ANF formation or to excess of nuclear antigen

In RA patients treated with gold salts at least once during the past few years the SSC titre of serum proved lower than in untreated ones This result essentially agrees with previous reports (see PERSELLIN HESS and ZIFF 1967) A similar tendency, though statistically non significant could be traced in the latex titres of serum and synovial fluid (Table XVIII) None of the RA patients studied was in complete remission the clinical activity of the disease was considered comparable in gold treated and untreated patients In sero positive RA (by the SSC test) the synovial fluid C activity was non significantly lower in gold treated than in untreated patients As a consequence of the mentioned influence of gold therapy on the RF titre especially the SSC titre of serum the relation between synovial fluid C′ activity and RF titre in RA could be strictly eval

urated only in patients not treated with gold salts during the years preceding examination. Below, such patients are referred to as 'untreated'.

Within the group of 71 untreated RA patients of the present study a negative correlation was established between the synovial fluid C' activity on the one hand and each of the three RF titres determined on the other side (the SSC titre of serum and the latex titres of serum and synovial fluid). This means that when the RF titre was markedly elevated the synovial fluid C' was generally markedly suppressed. On the other hand when the RF titres in RA were negative the synovial fluid C' activity was high or non-suppressed and essentially comparable to that found in various sero-negative non-RA arthropathies such as uro-polyarthritis, ankylosing spondylitis, psoriatic arthropathy and (juvenile) oligoarthritis (ANSELL and BYWATERS 1962).

The findings in juveniles and adults were very similar, especially as regards the relation between RF titre and synovial fluid C' activity (Appendix Tables V and III—IV). For this reason no distinction was made between juvenile and adult arthropathies.

In the present series of various arthritides as well as in RA as a whole no relation was found between the synovial fluid C' activity values and the intensity of synovitis nor between these values and the time for previously given intra-articular injection or other therapeutic measures employed (corticosteroids *per os*, ACTH, salicylate, phenylbutazone, chloroquine etc.). In steroid-treated as well as in untreated RA no relation was found between the synovial fluid C' activity and the ESR (Table XVI).

It should be noted that among the 100 patients accepted as RA only 13 had shown consistently negative RF tests. Five out of these 13 were juveniles with a fairly early onset of joint symptoms (on an average at about 4 1/2 years), where sero-negativity is the rule (BYWATERS, CARTER and SCOTT 1959, SIEVERS, AHVONEN, AHO and WAGER 1963).

The inverse relationship between the RF titre of serum and the synovial fluid C' activity was perhaps most clearly manifest in the group of (untreated) RA patients with subcutaneous nodules in relation to RA patients (untreated) without nodules: these patients—with nodules—showed significantly higher RF titres of serum and highly significantly lower synovial fluid C' activities (Table XXI). The RF titre of synovial fluid (by the latex test) however was essentially comparable in the two RA groups compared (Table XXI). The higher RF titres of serum in RA with nodules agree with previous reports (SIEVERS 1965 and others).

As mentioned already, the results in normal subjects and in most instances of effusion in cases of sero-negative non-RA arthropathy indicated that whole C' of serum passed through the capillaries and the subsynovial and synovial tissues into the joint space without being appreciably changed. In cases of sero-positive arthropathy, on the other hand, suppressed and some

times extremely suppressed synovial fluid C′ activities were observed These results indicate a local binding of C′ as a possible cause of the suppression of the synovial fluid C′ activity in sero positive arthropathies

It is known that gamma globulins, aggregated (denatured) by means of heat treatment or by chemical treatment exert a strong anticomplementary activity (OHACEN 1945 ISHIZAKA and ISHIZAKA 1960, FROMMHAGEN and FUDENBERG 1962) It has been suggested that the interaction between antigen and antibody is accompanied by a structural change (denaturation) of the antibody molecule which is presumably the pre requisite for C′ fixation (ROBERT and GRABAR 1957 ISHIZAKA and CAMPBELL 1959) Physically denatured I_gG and antigen antibody complexes fix C′ components in the same sequence and to essentially the same extent (ISHIZAKA ISHIZAKA and BORSOS 1961)

Evidence for C′ being *in vivo* bound to the rheumatoid synovial membrane has been presented by RODMAN, WILLIAMS BILKA and MÜLLER EBERHARD (1964) By means of immunofluorescence technique two C′ components (β_{IC} and β_{IE}) could be demonstrated in rheumatoid synovial membrane although not in normal one The localization of these C′ components corresponded most closely with deposits of IgG Similar results have since been reported by FISH MICHAEL GEWURZ and GOOD (1966) These studies suggest that the synovial membrane deposits of IgG contained IgG in an aggregated form and that *in vivo* binding of C′ had appeared for this reason Previous studies by KAPLAN and VAUGHAN (1962) also suggested that the synovial membrane deposits of IgG which were more marked in RA than in other arthropathies studied, contained IgG in an aggregated form

Of the classical C′ components studied in rheumatoid synovial fluid, C′1 and C′4 (β_{IE}) have been found to be suppressed (ZVAIFLER and PEKIN 1963 FOSTIROPOULOS AUSTEN and BLOCH 1965), in addition the latter authors found C′2 to be low too

MÜLLER EBERHARD and NILSSON described (1960) a conversion product (β_{IA}) of β_{IC} which could be identified by means of immuno electrophoresis A distinct β_{IA} line however seemed to be a rare phenomenon in rheumatoid synovial fluid only one out of 18 such fluids showed a distinct β_{IA} line (HEDBERG unpublished observations)

When examining synovial fluid for β_{IA}, it was found that several fluids with extremely suppressed C′ activities lacked β_{IC} in amounts detectable by the immune serum used Estimation by OUCHTERLONY technique proved the concentration to be low (HEDBERG unpublished observations) This result has subsequently been confirmed by quantitative methods (LUNDH 1965 b)

The location of two C′ components (β_{IC} and β_{IE}) in the rheumatoid synovial membrane both of which are present in low concentrations in synovial fluid with a suppressed total C′ activity supports the idea of a local binding of C′ as a cause of the low synovial fluid C′ activity

Most authors nowadays consider RFs to be antibodies directed against slightly altered or denatured IgG (see review by KUNKEL and WILLIAMS 1964). Results published by MELLORS, HEIMER, CORCOS and KORNGOLD (1959), MCCORMICK (1963) and BRAUNSTEINER, RIEGLER and PAKESCH (1962), suggest that RFs are synthesized by plasma cells at various sites such as the inflamed synovial membrane, lymph nodes, subcutaneous nodules and bone marrow.

KAPLAN (1963) has suggested that the formation of RFs is initiated by the presence in rheumatoid tissue lesions of IgG in an aggregated form. The above mentioned evidence for C′ components being bound to probably aggregated IgG in rheumatoid synovial membrane combined with the inverse relationship between synovial fluid C′ activity and RF titres demonstrated in the present study consequently support KAPLAN's hypothesis for the RF formation.

In short, the relationship between a suppressed synovial fluid C′ activity and an elevated RF titre seems compatible with the assumption that both phenomena are to some extent secondary to the presence of denatured IgG, i.e. in the inflamed synovial membrane. This hypothesis, which is closely related to that of KAPLAN (1963), was adopted by the author.

In a study dealing with the characteristic sero-negativity in patients with psoriatic arthropathy, LASSUS, MUSTAKALLIO, LAINE and WAGER (1964) suggested the presence in such cases of some factor preventing the formation of macroglobulins including RF and/or promoting their breakdown. The high and non-suppressed synovial fluid C′ activity found in psoriatic arthropathy involving the distal interphalangeal joints suggests that here—as in other sero-negative non-RA arthropathies[1]—the stimulus for the formation of RFs is essentially lacking, at least in the inflamed synovial membrane.

Suppressed synovial fluid C′ activities in psoriatic arthropathy were observed among the (four) doubtful cases where involvement of the distal interphalangeal joint was lacking. Three out of these four patients showed suppressed synovial fluid C′ activity, and two of these three detectable RF activity in synovial fluid, whereas all four showed entirely negative serum tests for RF. In the series of patients with psoriatic arthropathy studied by LASSUS, MUSTAKALLIO, LAINE and WAGER (1964), elevated RF titres (of serum) were found almost exclusively among cases where the distal interphalangeal joints were not involved. The frequency of elevated RF titres was, however, relatively low (about 30 per cent).

Attempts to demonstrate immunoglobulins and C′ (as β_{1C}) in the skin lesions of psoriasis and various other dermatoses have failed (KALSBEEK and CORMANE 1964). By way of contrast, in SLE IgG and β_{1C} have been demonstrated to be concomitantly present in

[1] Against a coincidence of RA and psoriasis argued the entirely negative serum tests for RFs combined with the absence of a typical RA distribution of the joint changes.

the skin lesions (KALSBEEK and CORMANE 1964) as well as in visceral lesions (LACHMANN, MÜLLER-EBERHARD, KUNKEL and PARONETTO 1962, PARONETTO and KOFFLER 1965). Several authors have emphasized that the concomitant presence of C′ components and immunoglobulins in a tissue lesion suggests, but does not prove, that C′ is bound because of a local antigen-antibody reaction (see for example LACHMANN et al. 1962). A denaturation of IgG not induced by impact of a serologic reaction should be expected to result in fixation of C′ not distinguishable from that induced by such an impact.

DAVIS (1966) suggested a common mechanism involving the C′ system in RA and SLE. Several observations however argue that the mechanism(s) underlying the activation of the C′ system in RA can be different from that in SLE. For example, the C′ level of serum is low in active SLE and generally raised in active RA (for refs. see DAVIS 1966). The reverse response of the C′ level of serum in RA (decrease) and in SLE (increase) after administration of ACTH (VAUGHAN, BAYLES and FAVOUR 1951 a, 1951 b) also suggests that different mechanisms can be *operating in the two syndromes*.

The results of the present limited series of SLE and SLE-like syndromes where the synovial fluid C′ activity was more consistently suppressed than in RA as a whole, suggested that the C′ system was early involved during the course of joint exudation (Table XIII).

In RA a suppression of the total C′ activity of synovial fluid seemed to be a late phenomenon in the present study as well as in that of PEKIN and ZVAIFLER (1964). These observations agree with the assumption that the deposits of IgG in early rheumatoid synovitis (KAPLAN 1963) predominantly consist of non-aggregated IgG. In agreement with this assumption are the observations that in early rheumatoid synovitis, in contrast to long-lasting, IgM (KAPLAN 1963) as well as RF synthesizing plasma cells (MELLORS 1963) could scarcely be demonstrated—presumably because the stimulus for RF formation in the form of aggregated IgG was weak or lacking.

Differences in sedimentation constants and precipitability in the cold between the RFs in SLE and those in RA have been demonstrated (SVARTZ and SCHLOSSMAN 1957, BLACK, GOLDIN, POSKE and MALMED 1959, SVARTZ and HEDMAN 1963). Most authors have reported elevated RF titres in 30—40 per cent of SLE sera. LEONHARDT (1964) found an even lower incidence.

In SLE associated with arthritis, on the other hand, as well as in SLE-like syndromes where arthritis is a more prominent feature, the RF titres tend*ed to be higher and more often ele*vated (LEONHARDT 1964 and others). In conformity with these findings was the relatively high incidence of elevated RF titres in the present limited series of SLE and SLE-like syndromes where all patients had arthritis. In one case of typical SLE (no gold therapy) entirely negative RF tests were combined with extremely sup-

pressed synovial fluid C′ activity (fresh effusion of 3 days duration). In this case the arthritis gradually subsided, the SSCs test remained negative at re-examination 3 years later.

In the whole series of 125 patients with various inflammatory arthropathies not treated with gold salts during the years preceding examination only 5 showed entirely negative tests for RFs combined with suppressed synovial fluid C′ activity. Out of these 5 patients only one had RA (juvenile cases of SSC neg. RA with onset of joint symptoms at 9 years), one was a case of typical SLE just mentioned and three were cases of probable psoriatic arthropathy, one of the latter showed a non suppressed C′ activity when re-examined. These exceptions to the rule of a connection between the RF titre and the synovial fluid C′ activity merely suggest that different mechanisms resulting in suppression of the synovial fluid C′ activity can be operating.

The other type of exception to this rule, i.e. that of a non-suppressed synovial fluid C′ activity combined with elevated RF titres, seemed to appear with some preponderance in instances of early effusion in RA without nodules (Fig. 8). However, also in early effusion a connection was found between the RF titre and the synovial fluid C′ activity if the whole series of various arthritides was considered (Fig. 8).

Because of the established relation between the RF titre and the synovial fluid C′ activity, the antibody nature and *in vitro* reactivities of RFs will be briefly considered below.

In human sera different types of anti-IgG antibodies may be discerned. KUNKEL and TAN (1964) listed 4 main types of which RFs and anti-antibodies in MILGROM's sense (MILGROM, DUBISKI and WOZNICZKO 1956) are of specific interest as regards the interpretation of the phenomenon of suppressed synovial fluid C′ activity.

Anti-antibodies in MILGROM's sense, in contrast to RFs, react only with IgG denatured by the impact of serologic reaction, not with native or physically denatured IgG; they are also rare in human sera and hardly ever seen in RA sera (MILGROM, DUBISKI and WOZNICZKO 1956, ANDRESEN 1963, MILGROM 1963 b).

Serum factors resembling human RFs can be induced experimentally in rabbits by immunization with physically denatured autochthonous IgG in FREUND's adjuvant (MILGROM and WITEBSKY 1960 a, MCCLUSKEY, MILLER and BENACERRAF 1962, CATSOULIS, ROTSCHILD, ORATZ and FRANKLIN 1965).

In most of the mentioned experiments RF-like substances were formed which showed preferential reactivity with xenogeneic IgG. In this respect as well as in sedimentation constant—7S instead of 19S—the RF-like substances differed from human RFs (KUNKEL and TAN 1964, CATSOULIS, ROTSCHILD, ORATZ and FRANKLIN 1965).

RF-like substances induced by immunization with papain split autochthonous IgG (WILLIAMS and KUNKEL 1963) were also predominantly of the

7S type although their reactivity against allogeneic (rabbit) IgG was comparable to that against xenogeneic (human)

Prolonged immunization of rabbits with foreign antigens such as *E coli* and *B subtilis* results in the appearance of RF like substances of a macroglobulin type which react primarily with allogeneic denatured IgG (ABRUZZO and CHRISTIAN 1961) Similar results have been reported by AHO and WAGER (1961) who used ovalbumin as an immunizing agent These anti IgG antibodies showed cross reactivity with human IgG Immunization of rabbits with β haemolytic streptococci, however resulted in the appearance of RF like substances reacting much stronger with xenogeneic than with allogeneic IgG (MILGROM and WITEBSKY 1960 b) The stimulus for the formation of the RF like antibodies was assumed to be denatured autochthonous IgG in these experiments (with foreign antigens) denatured by the impact of a serologic reaction

It is not clear from the above mentioned experiments which—if any—of the experimentally induced RF like substances should be considered true counterparts to human RFs (KUNKEL and TAN 1964) Yet MILGROM (1964) as well as KUNKEL and TAN (1964) were inclined to consider the RF like substances induced by immunization with bacteria as most closely related to human RFs

RF activity is directed against those papain fragments of human (fragment F) and rabbit (fragment III) IgG which lack antibody combining site (FRANKLIN 1961, GOODMAN 1961 and others) Anti antibodies, on the other hand are directed against those papain fragments of human and rabbit IgG on which the antibody combining site is located (FUDENBERG GOODMAN and MILGROM 1964)

The RF like substances induced by hyperimmunization of rabbits with bacteria were directed against the 'non antibody' fragment of rabbit IgG (fragment III) (CHRISTIAN 1963)

The Gm(a) and Gm(b) sites are contained in the non antibody fragment of human IgG (FRANKLIN FUDENBERG MELTZER and STANWORTH 1962, HARBOE OSTERLAND and KUNKEL 1962)

In view of the above mentioned data and some pertinent observations of Gm(f) GRUBB KRONVALL and MÅRTENSSON (1965) stated that immunological findings in RA draw attention to the F piece (the non antibody fragment) of IgG As mentioned the suppression of the synovial fluid C activity is interpreted as essentially being secondary to binding of C to presumably aggregated IgG in the rheumatoid synovial membrane If this interpretation is correct it is of interest that in the non antibody fragment of human (and rabbit) IgG is also contained the C' fixation site (as well as the skin irritating capacity of aggregated IgG) (TARANTA and FRANKLIN 1961 TARANTA FRANKLIN and OVARY 1962 ISHIZAKA 1963)

It should be noted that unaggregated papain fragments of IgG capable of inducing formation of RF like substances when injected in rabbits (WIL

LIAMS and KUNKEL 1963), were in themselves unable to fix C′ (ISHIZAKA 1963) Nor are the reactions between RFs and the non antibody fragment of human IgG (see review of KUNKEL and TAN 1964) or between RFs and aggregated human whole IgG (HEIMER and LEVIN 1965) accompanied by a demonstrable fixation of C′

Experimental evidence has been presented (BUTLER GLEICH and VAUGHAN 1962 and others) to show that RFs react primarily with human IgG and that part of the RFs also cross reacts with animal IgG In RA this is reflected by a higher degree of sensitivity of the latex test as compared with that of the SSC test (AHO 1961 SIEVERS 1965 and others) The result was similar in the present study although the two tests here were not strictly comparable because the latex titre was determined in the euglobulin fraction of serum and synovial fluid and the SSC titre in whole serum

It is of interest that at clearly negative SSC titres of serum (below 32) where the higher sensitivity of the latex test was most evident the occurrence of elevated latex titres in serum or synovial fluid was almost exclusively confined to patients with a suppressed synovial fluid C′ activity (Table XX) This result suggests that a response to denatured autologous IgG is more easily detected by a test system where allogeneic IgG is the reactant *i e* the latex system than by a test system where xenogeneic (rabbit) IgG is the reactant *i e* the SSC system As already mentioned such a reactivity pattern of human RFs fits in most closely with that of the RF like substances induced by immunization with bacteria

In the whole series of sero positive arthropathies the RF titre of synovial fluid by the latex test was on an average comparable to that of serum Slight to moderate deviations were essentially found equally often in both directions (Table XXII) Similar results have been demonstrated in other series (*e g* RODNAN EISENBEIS and CREIGHTON 1963) In RA with nodules and extremely low synovial fluid C activities the latex titre of synovial fluid tended to be low in relation to that of serum (Table XXII) The data presented by FOSTIROPOULOS AUSTEN and BLOCH (1965) pointed in the same direction although the titre differences were more pronounced This was perhaps a manifestation of the affinity of RF as well as of C to denatured IgG (DAVIS and BOLLET 1964 MILGROM and SCHULTZ 1965 and others) both having possibly been bound to denatured IgG during the passage through the synovial and subsynovial tissues

In the present study however a similar tendency towards a low latex titre of synovial fluid in relation to that of serum was observed in instances of early effusion in RA without nodules in these cases often combined with a non suppressed synovial fluid C activity (Table XXII) The interpretation of this result is difficult one possibility is that in these instances of early effusion in RA the permeability of the periarticular tissues for RFs was reduced Another possibility is affinity between RFs and (probably) native IgG However because of the methodological errors involved in determinations of the latex titre too much importance should not be attached to the above mentioned doubtful differences between the latex titre in serum and that in synovial fluid

Evidence suggestive of an *in vivo* affinity of RFs as well as of C′ to presumably aggregated IgG has recently been presented by FISH GEWURZ and

GOOD (1966) synovial membrane specimens from some RA patients showed (immuno-fluorescence technique) deposits in the same area of IgG and C′ (as β_{1C}) whereas specimens from other RA patients showed deposits of IgG and RF. In more than half the number of the RA patients the deposits of C′ and IgG had a focal distribution. Because of the latter observation and the lack of knowledge about the mechanism(s) underlying exudation in RA, it is not possible to decide to which extent the C′ activity of synovial fluid is representative of the synovial and subsynovial tissues as a whole. At present it can merely be assumed that a suppression of the synovial fluid C′ activity essentially reflects the occurrence in these tissues of free denatured IgG not previously blocked by C′ and/or by RFs.

The extent to which various sites of RF formation contribute to the total activity of the pool of circulating RFs is entirely unknown.

The extremely suppressed synovial fluid C′ activity in most instances of markedly elevated RF titres suggests that in RA aggregated IgG can be continuously formed in considerable amounts in the inflamed rheumatoid synovial membrane. If this is true, the extremely low synovial fluid C′ activities suggest, as the titres do, that the stimulus for RF formation can be very strong.

Especially at moderately elevated SSC titres it was found in some patients that synovial fluid from one joint showed a very low C′ activity, whereas fluid from another joint showed a very high C′ activity. It is not known whether or not this sometimes considerable intra-individual variation of the synovial fluid C′ activity could to any extent be ascribable to a situation where all aggregated IgG was blocked and hence no C′ was trapped during the passage through the synovial membrane. Presumably, as suggested above, the degree of suppression of the synovial fluid C′ activity can be interpreted as a function of the rate by which free (non-blocked) aggregated IgG is formed in the synovial membrane. In any case the observed synovial fluid C′ activity was found to be on an average lower at moderately elevated RF titres than at entirely negative ones.

Among untreated RA patients with negative RF titres in serum and synovial fluid only one out of 15 (11 adults and 4 juveniles) showed suppressed synovial fluid C′ activity. As already mentioned this occurred in a girl with onset of joint symptoms at 9. The incidence of 1/4 of a suppressed synovial fluid C′ activity in the present limited series of sero-negative juvenile RA was not distinct from that of 3/7 reported by FISH, MICHAEL, GEWURZ and GOOD (1966). The almost consistently non-suppressed synovial fluid C′ activity in (untreated) RA with negative RF tests supports the idea that here stimulus for RF formation in the form of aggregated IgG in sufficient amounts in the synovial and subsynovial tissues was essentially lacking. The same is true of the whole group of various sero-negative arthritides (Fig. 2) except for the doubtful cases of psoriatic arthropathy, where, as mentioned, elevated RF titres are sometimes found.

According to the hypothesis that suppressed synovial fluid C′ activity essentially reflects the presence in the

synovial and subsynovial tissues of stimulus for RF formation in the form of aggregated IgG a suppression of the synovial fluid C′ activity during an early sero negative phase of RA is expected to precede the appearance of a positive RF test The present study does not shed light on the question of whether or not this is the case the results seemed to point in the expected direction only in one RA patient where synovial fluids from both knee joints were studied early during the exudation In this patient, however the suppression of the synovial fluid C activity was transient (Fig 9)

It is hard to explain the reason for the frequently negative SSC titre of serum combined with elevated latex titres and suppressed synovial fluid C′ activity in patients treated with gold salts during the years preceding examination This finding is compatible with one or more of the following explanations a modified denaturation of IgG resulting in essentially unaffected formation of RFs with in vitro reactivity against human IgG but no detectable formation of RFs with reactivity against rabbit IgG (in the SSC test) or a failure to respond to stimulus for formation of RFs with reactivity against rabbit IgG (in the SSC test) or an increased elimination of formed RFs with reactivity against rabbit IgG Recent observations by PERSELLIN HESS and ZIFF (1967) argue against the second alternative they found that intense gold treatment did not influence various types of immune response *in vivo* such as formation of humoral antibodies (rabbits) and delayed hypersensitivity (guinea pigs) Nor was the induction of RF like substances affected

As already mentioned previous reports failed to demonstrate a clear cut relation between the synovial fluid C′ activity and the RF titre in RA In view of *ia* the considerable inter and intra individual variation of the synovial fluid C′ activity at different RF titre levels (except for the highest SSC titres) and the influence of previous gold therapy on the RF titre especially the SSC titre it seems conceivable to ascribe this failure to the series of RA having been too small

The raise of the C′ level of serum in wide spread inflammatory processes can be interpreted as a non specific reaction resembling the appearance of various acute phase reactants in blood plasma (FISCHEL 1953) Although the results concerning the C level of serum in RA were far from clear it is worth mentioning that in untreated RA the result agreed with such an interpretation the C level of serum correlated with the ESR (SPEARMAN s r = +0 32 *) The findings in a group of various (untreated) non RA sero negative arthropathies were similar (SPEARMAN s r = +0 40*)

Steroid treated and untreated RA with extremely suppressed synovial fluid C activities (values below 25) showed mean C levels of serum lower than those for corresponding non RA sero negative arthritides ($p < 0.05$) The means of the ESR in the groups compared were comparable The result in untreated patients with SLE or SLE like syndromes was similar

In contrast to untreated RA, steroid treated RA showed no correlation between the C′ level of serum and the ESR (SPEARMAN's $r = +0.07$). In this group (of steroid treated RA) a highly significant degree of correlation was found between the C′ level of serum and the synovial fluid C′ activity (SPEARMAN's $r = +0.56^{**}$).

The results mentioned were compatible with an increased C′ consumption reflected in a low C′ level of serum. Other possibilities, however, can not be ruled out, for example anticomplementary activity and an inhibited synthesis of one or more of the C′ components. In addition no correlation was found in untreated RA between the C′ level of serum and the synovial fluid C′ activity.

LAURELL and GRUBB (1958) found the titres of the classical C′ components to be essentially normal in RA sera. As mentioned, some of these (C′1, C′2 and C′4) as well as β_{1C}, a moiety of the classical C′3, have been showed to be suppressed in rheumatoid synovial fluid.

LUNDH (1965 a) found that in conditions other than active SLE, RA and acute glomerulonephritis β_{1C} behaved essentially like an acute phase reactant. In RA, however, the β_{1C} concentration in serum was normal rather than raised. This observation together with the often low concentration of β_{1C} in rheumatoid synovial fluid fits in with the idea that a local consumption of this component is a cause of the relatively low serum concentration.

Summary (part I)

1. Relative values of the C′ activity of synovial fluid rather than the number of C′ units were used.* This made a comparison between patients or groups of patients possible.
2. The essential finding of the present study was the establishment within the fairly large group of RA of a highly significant degree of negative correlation between *the synovial fluid C′ activity* and the RF titres of serum and synovial fluid.
3. In RA with negative RF tests at the time of examination the synovial fluid C′ activity was found to be only non-significantly lower than that in a miscellaneous group consisting of the above mentioned non-RA sero-negative arthritides. This refers specifically to patients not treated with gold salts during the years preceding examination. In gold treated patients, where the SSC titre of serum (though scarcely the latex titres of serum and synovial fluid) seemed to be modified downwards, several patients showed suppressed synovial fluid C′ activities combined with a clearly negative SSC test.
4. In instances of a clearly negative SSC test in gold treated and untreated patients the latex titres of serum and/or synovial fluid were found to be elevated almost exclusively when the synovial fluid C′ activity was suppressed.
5. In relation to RA without nodules RA with subcutaneous nodules showed highly significantly lower synovial fluid C′ activities and *significantly higher RF titres of serum*.
6. Like a small group of SLE and SLE-like syndromes, RA as a whole showed on an average lower synovial fluid C′ activities than did various non-RA sero-negative arthritides such as ankylosing spondylitis, uro-polyarthritis, psoriatic arthropathy and oligoarthritis (juvenile).
7. In the small group consisting of a few typical cases of SLE and predominantly of SLE-like syndromes the synovial fluid C′ was

* The relative synovial fluid C′ activity was defined as $(C_{SF} \times 1000)/(C_S \times TP_{SF})$, where C_{SF} and C_S stands for the number of C′H_{50} units per ml synovial fluid and serum respectively, and TP_{SF} for the protein content of synovial fluid in g per cent.

on an average extremely low and comparable to that found in RA with nodules In this group too, low synovial fluid C′ activities were in most cases combined with elevated RF titres

8 The results tended to show that a suppression of the C′ activity of synovial fluid was a late phenomenon in RA and probably an early one in the group of SLE and SLE like syndromes

9 The C activity of *synovial fluid* showed no clear relation to parameters other than the RF titre, such as anticomplementary activity, white blood cell counts of synovial fluid and the concentration of gamma globulins of synovial fluid nor to the intensity of synovitis the ESR and antinuclear activity of serum

10 The result as regards the fairly close association between the phenomenon of a suppressed synovial fluid C′ activity and the phenomenon of elevated RF titres was discussed Both phenomena may *be secondary to the presence of* deposits of aggregated (denatured) IgG in the synovial membrane

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II THE OCCURRENCE OF MONONUCLEAR CELLS WITH *IN VITRO* CYTOTOXIC EFFECT

Introduction

Under appropriate conditions experimentally induced autoimmune diseases such as allergic encephalitis (EAE) (PATERSON 1960 STONE 1961, ÅSTRÖM and WAKSMAN 1962) and nephrosis (HESS ASHWORTH and ZIFF 1962 HEYMANN HUNTER HACKEL and CUPPAGE 1962) can be transferred by means of immune lymphoid cells[1] whereas transfer with immune serum has failed. Successful attempts to transfer experimental autoimmune *thyroiditis with lymphoid cells have* been reported by some authors (FELIX DAVIES and WAKSMAN 1961 GOODMAN 1962 KOFFLER and PARONETTO 1965) whereas others have failed (ROSE KITE DOEBBLER and BROWN 1963) Similarly transplantation immunity can be passively transferred by immune lymphoid cells (for refs see GOWANS 1965) WAKSMAN and WENNERSTEN (1963) were able to transfer experimental adjuvant arthritis by means of lymphoid cells

These successful transfer experiments principally similar to the transfer of delayed hypersensitivity by means of immune lymphoid cells first described by LANDSTEINER and CHASE (1942) and CHASE (1945) demonstrated the pathogenetic role played by the lymphoid cells However humoral factors *e g* humoral antibodies produced by the transferred living lymphoid cells (see HARRIS 1965) might have mediated the tissue damage (KRETSCHMER and PEREZ TAMAYO 1962 KARUSH and EISEN *1962*) Evidence against as well as in favour of involvement of humoral factors—derived from the transferred lymphoid cells—has been reported (see NAJARIAN and FELDMAN 1962 1963 KRETSCHMER and PEREZ TAMAYO *1962*)

Lymphoid cells from immunized animals have been shown to be capable of inducing cytotoxic effects on tissue cultures of the cells against which the animals had been immunized For example in EAE immune lymphoid cells have been shown to exert a cytotoxic effect on cultured glia cells (KOPROWSKI and FERNANDES 1962 BERG and KALLEN 1963) similar *in vitro* cytotoxic effects have been described in experimentally induced autoimmune neuritis (WINKLER 1965) and autoimmune nephrosis (HOLM 1966

[1] Lymphoid cells or mononuclears refer to such cells of various sources such as spleen lymph node thoracic duct and peripheral blood Immune lymphoid cells derived from immunized animals

1967) and also in experimental auto immune thyroiditis (ROSE KITE DOEBBLER and BROWN 1963 BJÖRKLUND 1964) In transplantation immunity similar observations have been made by GOVAERTS (1960) and WILSON (1963), whereas some other attempts to demonstrate *in vitro* cytotoxicity have failed (for refs see WILSON 1963)

In a series of other experimental conditions, immune lymphoid cells have been shown capable of exerting cytotoxic effects on various normal and neoplastic target cells in tissue culture (ROSENAU and MOON 1961 1964, 1966 ROSENAU 1963 BRONDZ 1964 MÖLLER 1965a b WILSON 1965a and others)

Most authors mentioned above observed only slight damage to cultured target cells when these were exposed to non immune lymphoid cells However moderate to strong cytotoxic effects (on xenogeneic or allogeneic target cells) have been reported *e g* by GINSBURG and SACHS (1965) and by HOLTZER (1966) HOLM and PERLMANN (1965) using a quantitative technique for the determination of the cytotoxic effect found xenogeneic non immune lymphoid cells to be moderately cytotoxic whereas allogeneic as well as syngeneic non immune lymphoid cells only occasionally exerted a slight cytotoxic effect The latter study was made with various combinations of lymphoid cells and target cells from rats and mice In a subsequent investigation by HOLM and PERLMANN (1967) normal human blood lymphocytes were studied for their cytotoxic effect on allogeneic liver cells on an average only a slight cytotoxic effect was observed (incubation time 24 hours) Essentially similar results have been reported when normal human blood lymphocytes were added to allogeneic fibroblast cultures (HIRSCHHORN FIRSCHEIN and BACH 1965, MÖLLER BECKMAN and LUNDGREN 1966) Nevertheless by prolonged exposure (2 to 7 days) of the human fibroblast cultures to allogeneic blood lymphocytes strong cytotoxic effects appeared under this condition even autochthonous lymphocytes were often found to be cytotoxic (MÖLLER BECKMAN and LUNDGREN 1966)

The introduction of *in vitro* models has made studies also on clinical materials possible (see review by ROITT and DONIACH 1967) It is of interest that in multiple sclerosis blood mononuclears have been shown to exert a cytotoxic effect on cultured glia cells comparable to that previously described in LAE (BERG and KALLEN 1964) In HASHIMOTO's thyroiditis LING ACTON ROITT and DONIACH (1965) were unable to demonstrate that blood mononuclears exerted a cytotoxic effect on cultures of thyroid cells Their result with lymphoid cells in experimental autoimmune thyroiditis which differed from those of ROSE et al (1963) and BJÖRKLUND (1964) were also negative

In ulcerative colitis PERLMANN and BROBERGER (1963) have demonstrated that blood mononuclears are capable of inflicting a cytotoxic effect on tissue cultures of colon cells This result has

been confirmed by WATSON, QUIGLEY and BOLT (1966).

The present investigation was designed as an approach to the question of whether or not lymphoid cells from arthritis patients can exert an *in vitro* cytotoxic effect similar to that described above. It was assumed that lymphoid cells deriving from the inflamed tissues surrounding the joint space are less diluted in synovial fluid than in blood. For this reason, lymphoid cells separated from synovial fluid were studied.

Since our first reports on exudate mononuclears (HEDBERG and KALLEN 1964 a, b) additional observations have been made. In order to obtain a better survey the results on the whole series of patients will be reported below. In rheumatoid arthritis (RA), BRAUNSTEINER, DIENSTL and EIBL (1963) had previously demonstrated a slight cytotoxic effect of lymph node cells on human amnion cells in tissue culture.

Materials and methods

The clinical material

Besides the selection principles described in Part I some additional criteria were required for the performing of cytotoxicity tests. One requirement was that the number of separated exudate mononuclears should be sufficient to make at least one test, i.e. $2.0 \pm 0.3^1 \times 10^6$ mononuclears. Because of the low recovery of mononuclears in the separation process a volume of synovial fluid of at least 10 ml, generally at least 20 ml, was required.

Another requirement was that the suspension of separated exudate mononuclears should contain at least 85 per cent mononuclear cells. The limit of 85 per cent was arbitrary.

If the recovery was too low or if the admixture with polymorphonuclears exceeded 15 per cent the initially accepted patient was excluded. This happened in about 1/3 of the cases intended for study.

The clinical material comprised 113 patients, 103 had adult and 10 juvenile forms of arthropathies. Out of the 113 patients 75 belonged to the series described in Part I. The patients were classified as described in Part I.

RA with nodules: 16 adults, 11 females and 5 males.

SSC pos. RA: 43 patients, 20 females, 19 males and 4 girls.

SSC neg. RA: 5 patients, 2 females both with negative latex tests, 2 males both with positive latex tests, and one boy with negative latex tests.

SLE and SLE like syndromes: 9 adults, 8 belonging to the series of Part I, these 8 cases are listed in Appendix Table VI as nos. 2—4 and 6—10. The added patient was a male with positive tests for LE cells and ANFs, the score according to LEONHARDT (1964) was 10.

Oligo arthritis (juvenile): one girl and two boys, two belonged to the series of Part I, the added patient was a boy, aged 8, with arthritis of knee joints alone. The RF tests were negative.

Ankylosing spondylitis: 11 patients (neg. RF tests), out of whom 9 were adult males, aged 28—51 (mean 37). Of the adults five had pronounced and four slight to moderate stiffness of the spine, all had X ray changes of the sacro iliac joints and seven had changes of the spine too.

Two had had juvenile forms with peripheral arthritis for 9 and 7 years respectively (onset of joint symptoms

[1] Standard deviation

at 9 and 12 respectively), both had X ray changes of the sacro iliac joints as well as of the spine (squaring of the vertebrae), they had slight to moderate pain of the back and slight limitation of movement the peripheral arthritis (highly active since onset with an essentially continuous course) involved more joints as compared with that in the adult patients although the finger joints were spared the RF tests on serum had been consistently negative for years and the latex test on synovial fluid was negative in both instances at the time of examination The peripheral arthritis was associated with but slight X ray changes

Uro polyarthritis (definition OLHAGEN 1960) 7 patients with an essentially acute form of the syndrome all males aged 17—41 (mean 21) four had a full and 3 an incomplete REITER syndrome, one of the latter and one of the former had a positive gonococcal C fixation test All had active prostato vesiculitis and negative RF tests —Included in this group was a male aged 25 with iritis and peripheral polyarthritis involving one knee one ankle and one sternoclavicular joint The arthritis had appeared in connection with a period of diarrhoea This patient, who had been ill for 4 months lacked signs of prostato vesiculitis The sacro iliac joints were normal

Osteoarthrosis 4 patients 3 females aged 51—62 and one male aged 59 All showed roentgenograms and synovial fluid cell counts compatible with the diagnosis

Gouty arthritis 1 female aged 71 with tophi and elevated uric acid in serum (several values above 8 mg per cent)

Psoriatic arthropathy 13 patients Twelve belonged to the series of Part I these cases are listed in Appendix Table VII as nos 1—2 4—6 and 8—14 The added patient was a male aged 51 with sero negative arthritis for years (no nodules) involving among others the distal inter phalangeal joints and the sacro iliac joints In four of the 13 patients the diagnosis was considered only probable because the distal inter phalangeal joints were not involved (see Part I) Below the 13 patients are considered together as one group of psoriatic arthropathy

Revision of the diagnosis

In three patients the diagnosis had to be revised Two females originally labelled atypical polyarthritis and SSC neg RA respectively subsequently developed psoriatic skin and nail changes and involvement of the terminal phalangeal joints the RF tests remained negative they were included in the mentioned group of psoriatic arthropathy

A third patient (male) originally labelled chronic uro polyarthritis (signs of active prostato vesiculitis sacro iliitis sero negative peripheral arthritis (for 7 years) confined to knees and wrists negative RF test) developed almost generalized arthritis transient nodules and positive RF tests a month after the initial examination this patient was included as SSC pos RA

Methods

The principle of the test system After treatment of the synovial fluid with

hyaluronidase[*] (120—150 viscosity reducing units/ml 37°C 15—20 min) and heparinization (about 50 IU/ml) the mononuclear cells were separated on cotton wool (WALKER and PALMER 1962) The white blood cells not adsorbed to the cotton wool were washed twice with PARKER 199 The suspension obtained was standardized to contain $2.0 \pm 0.3 \times 10^6$ cells/ml[**] As already mentioned only suspensions consisting of at least 85 per cent mononuclears were accepted

The standardized suspension of mononuclears was preserved at 37°C for a varying time (1/2 hour to about 4 hours) before 1 ml of the suspension (about 2×10^6 cells) was added to cultures of human skin fibroblasts

Primary cultures of human fetal fibroblasts were used Fibroblast cultures were initiated from explants of skin from abortuses 20—25 cm long Small pieces of non trypsinized skin were placed directly on the glass bottom of LEIGHTON tubes After 15—30 minutes a fluid medium was added consisting of 5 per cent chick embryo extract 20 per cent adult human serum and 75 per cent PARKER 199 and containing penicillin (50 IU/ml) and streptomycin (50 μ_g/ml) Good growth of spindle shaped fibroblasts was usually obtained within a few days and dense mats of fibroblasts soon formed The medium was changed regularly but cultures were not used for tests during the first 4 days after the last medium change Incubation 37°C No other temperatures were tried

The cultures were studied with phase contrast microscope Before adding of mononuclears (day 0) part of the culture showing good growth was selected and photographed, the suspension of mononuclears was added and incubated with the culture for 15—20 hours, the culture was rinsed with prewarmed PARKER 199 and a new photograph (day 1) of the same selected part of the culture was taken The culture was then incubated for a further 20—24 hours, this time with those mononuclears alone that had not been rinsed away If after this last incubation (day 2) the culture remained unchanged photographing was occasionally omitted —For further technical details the reader is referred to a previous report (HEDBERG and KALLEN 1964 a)

By comparing day 1 and day 2 photographs with the day 0 photograph the degree of cytotoxicity could be (roughly) estimated A simple score— "0" "+" and "+ +"—was used as shown and described in Figs 1—2 only a "+ +" reaction (on day 1 or day 2) was considered significant, in most instances of a "+ +" reaction this appeared on day 1 Cytotoxicity in this test system was defined as the capacity of the mononuclears to detach the fibroblasts from the glass

The initially selected field of the monolayer culture seemed to be essentially representative of the whole cul

[*] HYALAS® LEO kindly supplied throughout the study by A/B LEO Halsingborg Sweden This preparation is known to be free from proteolytic enzymes

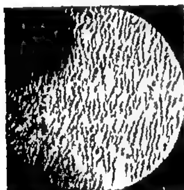
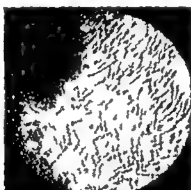
[**] In the first part of the study the suspension was standardized to contain $1-3 \times 10^6$ cells/ml



F o 1 Photog aphs of f brobla t cultures before add on of exudate mononuclears da 0 15—?0 hours later day 1 of the same m ro cop c f eld and after an add t onal ?0—?4 hours (day ?) Three cases of RA
C totox y score A 0 B 0 C

Day 0 | Day 1 | Day 2



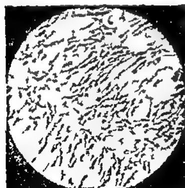




Fig. 2 Photographs of fibroblast cultures on day 0, day 1 and day 2 (see Fig. 1). Three cases of psoriatic arthropathy.
Cytotoxicity score for all three tests = + +

ture with respect to the outcome of the test, in some of the tests scored + minor variations seemed to occur in different parts of the culture when the tests were scored '0 or ++ no consistent difference between different parts of the culture was observed

In the first series of patients studied (HEDBERG and KALLÉN 1964 a b) the diagnosis of the patient was unknown when the test result was evaluated In the remainder corresponding to about half the number of patients (as well as in the repeated tests) the diagnosis was known The distribution of positive cytotoxicity tests over different arthritis groups was essentially one and the same in both cases When repeated tests were made the patient was represented by the first one made Some tests were made with the patient's whole serum or IgG present as described below The IgG fraction prepared from whole serum by chromatography on DEAE cellulose according to the technique described by SOBER GUTTER WYCKOFF and PETERSON (1956) was immunoelectrophoretically pure (see HEDBERG and KALLÉN 1964 b)

Trypan blue technique (ENGELFRIET and BRITTEN 1965) was used to estimate the viability of the mononuclears after separation and also in a few experiments for study of the fibroblasts which had been detached from the glass In a series of 25 consecutive determinations on an average 10 per cent of the separated mononuclears were found trypan blue positive (range for 24 determinations 3—16 per cent one alone showed 25 per cent) Similarly separated blood mononuclears consistently yielded less than 5 per cent trypan blue positive cells

The recovery of exudate mononuclears at the separation process amounted on an average to about 1 per cent As mentioned before a low recovery or admixture with polymorphonuclears fairly often made a cytotoxicity test impossible sometimes rapid coagulation despite heparinization made a separation of mononuclears impossible

The RF and ANF tests were those described in Part I The latex titre of synovial fluid was not consistently determined in this series of patients

CHAPTER I

Cytotoxicity tests with exudate mononuclears

a) Findings in various arthropathies

As mentioned above, only tests scored ++ were considered significant, whereas tests scored 0 or + were considered negative (see Materials and Methods).

Positive tests (score = ++) were found predominantly in SLE or SLE-like syndromes and in psoriatic arthropathy, some RA patients, especially those with nodules, and one of the patients with ankylosing spondylitis also showed a positive test (Table I).

Negative tests (scored 0 or in a few cases +) were obtained in 10 out of 11 patients with ankylosing spondylitis and in 8 with uro-polyarthritis (Table I). These 19 cases are considered as a reference group below. Four adults with osteo-arthrosis and one with gouty arthritis associated with osteo-arthrosis, three juveniles with oligo-arthritis and a considerable proportion of the RA patients (44 out of 64) also showed negative tests (Table I).

Among the 44 RA patients with negative tests a weak or doubtful test (scored +) was observed in three. Similar doubtful cytotoxic effects were observed in two out of 10 cases of ankylosing spondylitis. In the remaining patients, totally 69, all tests were scored 0.

The cytotoxic effect seemed to be preceded by clustering of the mononuclears ("contactual agglutination") on the surface of the fibroblasts (Fig. 3).

The above-mentioned weak cytotoxic effects scored + were associated with contactual agglutination. Contactual agglutination without detectable cytotoxic effect (score = 0) were noted in three cases alone (of RA) *.

In the group of SLE and SLE-like syndromes as well as in psoriatic arthropathy positive cytotoxicity tests were found highly significantly more often than in the reference group (Table I).

In RA as a whole the frequency of positive cytotoxicity tests was probably higher than that in the group of ankylosing spondylitis and uro-polyarthritis used as a reference ($p = 0.03$, Table I).

* Contactual agglutination in association with a cytotoxic effect as a routine studied only 15—20 hours after the addition of exudate mononuclears to the fibroblast cultures seemed to be somewhat more impressive in psoriatic arthropathy than in other arthritides where cytotoxic effects were found.

Table I Frequency of positive cytotoxicity tests (scored + +) with exudate mononuclears in various arthropathies

Diagnosis	No of cases	Frequency of pos tests		P*	
SLE and SLE like syndromes	9	9/9		0.000003	
Psoriatic arthropathy	13	10/13		0.00009	
RA with nodules	16	9/16		0.002	0.03
SSC pos RA	43	11/43		0.10	
SSC neg RA	5	0/5		—	
Ankylosing spondylitis	11	1/11	1/19	—	
Uro poly arthritis	8	0/8		—	
Osteoarthrosis	4	0/4		—	
Gouty arthritis	1	0/1		—	
Oligo arthritis (juvenile)	3	0/3		—	

* The frequency of positive tests was compared with that of 1/19

In SSC pos RA however the largest group of RA the results showed only a *non significant tendency towards a* higher incidence of positive cytotoxicity tests as compared with that of the reference group ($p = 0.10$ Table I)

Within the group of RA as a whole patients with subcutaneous nodules showed positive cytotoxicity tests significantly more often than did patients without ($p = 0.002$ Table I)

b) Cytotoxicity tests made with different fibroblast cultures as targets

Experiments were performed in which exudate mononuclears were simultaneously added to different fibroblast cultures (Table II) The cultures derived from three sources fetal and adult skin and adult rheumatoid synovial membrane

As shown in Table II the test results with the cultures of fetal skin agreed closely with those obtained with the other two types of (on an average older) cultures in one instance a weak cytotoxic effect (in association with contactual agglutination) was obtained with the culture of adult skin where no detectable cytotoxic effect was found in the culture of fetal skin (Table II)

Different fetal skin cultures of the same or different age and source also



(Score on day 2 = +)



(Score on day 2 = +)



(Sore on day 2 = + +)



(Sore on day 2 = + +)

Fig 3 Contactual agglutination on fibroblasts exposed for 7 1/2—13 hours to exudate mononuclears (4 patients)

Table II Results of simultaneous challenge of different fibroblast cultures with exudate mononuclears alone Aliquots of the suspension of mononuclears were simultaneously added to the different cultures
Figures in parenthesis = Age of the cultures in days
RA = Rheumatoid arthritis
PsA = Psoriatic arthropathy
0 = no cytotoxic effect
\+ = doubtful cytotoxic effect
\+ + = strong cytotoxic effect

Diagnosis	Source of fibroblast cultures			
	Fetal skin		Adult skin	Synovial membrane (rheumatoid)
RA	0 (2)	0 (9)	—[1]	—
PsA	+ + (5)	0 (5)	—	—
RA	0 (6)	0 (6)	—	—
PsA	+ + (7)	+ + (7)	—	—
RA	+ + (11)	+ + (17)	—	—
PsA	0 (14)	0 (21)	—	—
RA	+ + (28)	+ + (35)	—	—
PsA	+ + (7)	+ + (7)	+ + (52)	—
RA	+ + (9)	—	+ + (60)	—
RA	0 (13)	—	0 (65)	—
RA	0 (13)	—	+ (46)	—
RA	+ + (12)	—	—	+ + (39)
PsA	0 (19)	—	—	0 (23)
Discrepancy	Clear cut	1/8	—	—
	Doubtful	—	1/4	—

[1] Not done

yielded identical test results in all except one out of 8 similarly made tests (Table II)

When cytotoxicity tests were repeated with one day old mononuclears (preserved overnight at 37°C) and with one day older fetal fibroblast cultures the results were similar in 8 cases the mononuclears remained non cytotoxic and in five out of six cases of cytotoxic mononuclears these were found to retain their cytotoxicity over night

The test results when different fibroblast cultures were challenged with exudate mononuclears (Table II) as well as the test results in the whole series of patients (Table I) were independent of the ages of the cultures The variation of the density of the fibroblast mats when different cultures were simultaneously challenged

with mononuclears (Table II) was presumably similar to that when the primary tests on the whole series were made (Table I). No attempts were made to determine the ratio mononuclears : target cells.

c) Cytotoxicity tests with exudate mononuclears secured on different occasions from the same patient, and the test results in relation to various treatments

Treatment with corticosteroids *per os* or with ACTH (Table IV), like other conventional therapy, did not seem to influence the test results. This was true also of various combinations of therapeutic agents. Whether or not recently given intra-articular injections of corticosteroids might have had any importance for the test results will be considered below.

In 43 patients exudate mononuclears secured on two or more occasions were tested for their cytotoxicity; in all 73 additional tests were made in these patients (Table III).

In Table III the results of the additional tests can be compared with those of the primary tests. No distinction was made between mononuclears derived from the same joint and those from different joints of the same patient. No relation was found between the test results and the time between the different tests, nor was there any appreciable change of the clinical state (disease activity and intensity of synovitis) of the patient between the different tests.

In SLE or SLE-like syndromes the results of the additional tests agreed with those of the primary tests: consistently positive tests were obtained (Table III). Similarly, consistently negative tests were obtained in cases belonging to the group of 'miscellaneous arthropathies'* studied by repeated tests (Table III). In both these groups of patients, the results of the primary tests (Table IV) as well as those of the additional ones seemed to be unrelated to intra-articular injections given at least two weeks before examination. In SLE or SLE-like syndromes positive tests were observed 2—3 weeks after such injections.

In RA and especially in psoriatic arthropathy discrepant test results were observed (Table III).

In psoriatic arthropathy, where 10 out of 13 initial tests turned out positive, most tests were made with mononuclears derived from joints not injected for several months or from non-injected joints (Table IV). In two of the three cases of a negative test intra-articular injections had been given 3 and 6 weeks respectively before examination (Table IV).

In these two cases three new tests made at least 10 weeks after the last injection of corticosteroid turned out positive twice and doubtful once (Table III). The doubtful test made 5 months after the last injection like the strongly positive tests, though not the negative ones made after 3 and 6 weeks respectively, was probably associated with contactual agglutination.

* = Ankylosing spondylitis, uro-polyarthritis and osteoarthrosis.

Table III Results of two or more cytotoxicity tests made on different occasions with exudate mononuclears alone derived from one and the same patient (no distinction was made between mononuclears from the same joint and those from different knee joints)

Diagnosis	No of cases	Primary tests			Subsequent tests				Discrepancy[1]		
		Cytotoxicity score			No of tests	Cytotoxicity score			No	Doubtful	Clear cut
		0	+	++		0	+	++			
SLE and SLE like syndromes	5	—	—	5	9	—	—	9	5/5	0/5	0/5
Psoriatic arthropathy	6	2	—	—	3	—	1	2	0/2	1/2	1/2
		—	—	3	4	—	1	3	2/4	1/4	1/4
		—	—	1	4	3	—	1			
								Total	2/6	2/6 (0)[2]	2/6 (2)
RA with nodules	11	2	—	—	8	8	—	—	5/5	0/5	0/5
		3	—	—	4	4	—	—			
		—	—	6	7	3	1	3	2/6	1/6	3/6
								Total	7/11	1/11 (0)	3/11 (0)
SSC pos RA (11)		1	—	—	5	5	—	—	5/7	1/7	1/7
		5	1	—	6	4	—	2			
SSC neg RA (1)	12	—	—	2	7	—	—	7	3/5	1/5	1/5
		—	—	3	3	1	1	1			
								Total	8/12	2/12 (2)	2/12 (0)
Total RA	23	11	1	—	23	21	—	2	10/12	1/12	1/12
		—	—	11	17	4	2	11	5/11	2/11	4/11
								Total	15/23	3/23	5/23
Ankylosing spondylitis	4	3	1	—	7	7	—	—	3/4	1/4	0/4
Uro poly arthritis	2	2	—	—	2	2	—	—	2/2	0/2	0/2
Osteo arthrosis	3	3	—	—	4	4	—	—	3/3	0/3	0/3
								Total	30/43	6/43	7/43

[1] Clear cut doubtful and no = Differences scored + +, + and 0 respectively

[2] Figures in parenthesis indicate instances of discrepant test results where the weaker cytotoxic reaction appeared after more recently given intra articular injections of corticosteroid than did the stronger one

In two experiments with cytotoxic mononuclears it was found that $1-2\times10^6$ mononuclears per ml elicited a cytotoxic effect whereas 0.5×10^6 or less were inefficient

Cytotoxic mononuclears that were preserved at 37°C overnight retained their cytotoxicity in five out of six experiments

Heat treatment at 56°C for 30—60 min which rendered the mononuclears trypan blue positive extinguished the cytotoxic effect (5 experiments), as did heat treatment at 100°C for 1 min (1 experiment)

Freezing and thawing also extinguished the cytotoxic effect (three experiments)

Adding of fresh blood donor serum (diluted 1 2 to 1 4) or fresh guinea pig serum (diluted 1 10) resulted in extinction of the cytotoxic reaction Preliminary experiments suggested that the extinguishing effect of a fresh hypogammaglobulinaemic serum used as a source of C (in the dilutions 1 5 or 1 10) was less pronounced as compared with that of fresh guinea pig serum

Similarly when the tests were made in the presence of the patient s fresh serum (diluted 1 2 or in one case 1 4) the cytotoxic reaction was extinguished in 6 out of 8 RA patients in all 3 with psoriatic arthropathy and in 1 out of 3 with SLE or SLE like syndromes Serum in itself was non cytotoxic [1]

In most cases heat inactivation (56°C 30 min) did not modify the protecting capacity of fresh patient serum in 9 out of 12 cases heat inactivated serum provided protection Also blood donor serum retained its protective effect after heat inactivation Adding of a 6 per cent solution of human albumin (w/v) had no protective effect nor had the patient s (non cytotoxic) IgG fraction in amounts of 1 1 to 3 1 mg per ml in 10 out of 11 similar experiments

The absence of a detectable contactual agglutination in cases of a protective effect of serum suggested that serum can in some way interfere with the contactual agglutination step of the cytotoxic reaction

In 8 cases of RA with a negative cytotoxicity test fresh hypogammaglobulinaemic serum, diluted 1 10 or in one case 1 2, was added as a source of C′ to non cytotoxic exudate mononuclears No detectable cytotoxic effect appeared in the presence of added C′

Some tests were made with mononuclears combined with phytohaemagglutinin (PHA), an agent known to provoke strong contactual agglutination A purified preparation of PHA was used in an amount of 10 μg/ml which in itself was essentially without detectable cytotoxic effect [2]

[1] In this limited series there was no relation between the protective effect of serum and the ABO group of the patient Nor was there any relation between the results of the cytotoxicity tests and the ABO group

[2] The PHA preparation was kindly placed at my disposal by Dr Jan Borjeson Thirty μg per ml of the preparation exerted a moderate to strong cytotoxic effect on the cultured fibroblasts whereas 10 μg induced degeneration of only a few fibroblasts Mitosis of human lymphocytes (2×10^6 per ml) was initiated by 1—2 μg per ml of the preparation Three of the PHA tests (with exudate mononuclears) were made with DIFCO s PHA M

In 4 out of 5 experiments where primarily non cytotoxic exudate mononuclears were combined with PHA a strong cytotoxic effect scored + + appeared

ROSENAU (1963) studied the effect of polylysine another agent known to induce strong contactual agglutination This type of agglutination however, was not followed by any cytotoxic effect (allogeneic test system) Similarly, when polylysine[4] was added to primarily non cytotoxic exudate mononuclears in the present (allogeneic) test system no detectable cytotoxic effect appeared (5 experiments)

Identical results were obtained when instead of non cytotoxic exudate mononuclears non cytotoxic peripheral blood mononuclears were used (separated as described below) PHA aggregation (11 experiments) although not polylysine aggregation (3 experiments) yielded a strong cytotoxic effect

e) *Relation to laboratory and clinical data*

Low recovery of mononuclears in the separation process often too low for making a single test and/or admixture with polymorphonuclears exceeding 15 per cent seemed to be associated with one or more of the following factors (perhaps most predominant in long lasting effusion in RA) pronounced capsular thickening extremely turbid synovial fluid and fluid rich in clots The tests repeated in one and the same patient (Table III) were made almost exclusively in long lasting synovitis

The sometimes discrepant test results observed exclusively in instances of long standing effusion could not be clearly related to a variation of the above mentioned factors

The question must be left open whether or not the separation process which as mentioned yielded a low recovery of exudate mononuclears might have had any importance as a selection mechanism[7]

In RA the following tendencies in the results of the cytotoxicity test could be traced a tendency towards positive tests in instances of a rapidly developing effusion and in cases of instability of the knee joint The test results could not be clearly related to any of the following parameters the intensity of the synovitis the ESR (see Table IV) the white cell count or the *differential count of synovial fluid* — The test results in relation to duration of effusion will be reported in a following chapter

In the group of RA and in that of SLE and SLE like syndromes (Table V) no relation was found between the SSC titre of serum and the occurrence of positive cytotoxicity tests In RA

[4] Poly L lysine HCl M A (Mann Res Lab Inc N Y) was used in an amount of 1 μg/ml This amount in itself non cytotoxic elicited a strong contactual agglutination it did not inhibit the cytotoxic reaction when added to primarily cytotoxic mononuclears

[5] An incubation of 40—45 hours was used as a routine because initial studies suggested this time to be sufficient

[7] Attempts to separate the exudate mononuclears by glass wool filtration (BRANDT BÖRJESON NORDEN and OLSSON 1962) were unsuccessful

Table V Frequency of positive cytotoxicity tests with exudate mononuclears alone at different SSC titre levels

	No of cases	SSC titre in serum			
		≤16	32—64	128—256	512—1024
RA	64	1/12	8/18	7/22	4/12
SLE and SLE like syndromes	9	3/3	1/1	1/1	4/4
Psoriatic arthropathy	13	10/13	—	—	—

only a non significant tendency ($p = 0.09$) was found towards a higher frequency of positive tests when the SSC titre was 32 or higher than at lower titres (Table V) The results were similar if the latex titre was considered instead of the SSC titre

A positive test for ANFs in serum appeared in RA patients with a positive cytotoxicity test as often as in those with a negative one In both cases a positive test for ANFs appeared in about 1/3 of the patients The appearance of a positive test for ANFs (in RA) was most clearly related to the presence of subcutaneous nodules

SUMMARY In *various arthropathies* exudate mononuclears were tested for their capacity to elicit a cytotoxic effect on tissue cultures of human embryonic fibroblasts Non trypsinized primary cultures were used, only clear cut effects were considered significant

The test system yielded reproducible results essentially identical test results were obtained when fibroblast cultures of the same or of various origin and age were simultaneously challenged with exudate mononuclears

Positive cytotoxicity tests were found predominantly in SLE and SLE like syndromes (9/9) and in psoriatic arthropathy (10/13) Some RA patients (20/64), especially those with nodules (9/16), also showed positive tests

Negative tests were obtained in all but one of 19 cases of ankylosing spondylitis and uro polyarthritis Four cases of osteoarthrosis and three of juvenile oligo arthritis also showed negative tests

When the tests were repeated in one and the same patient consistently positive tests were obtained in the group of SLE and SLE like syndromes and consistently negative ones in ankylosing spondylitis uro polyarthritis and osteoarthrosis Some cases of RA and psoriatic arthropathy showed discrepant test results

No relation was found between the test results and the intensity of the synovitis or the disease activity as judged from the ESR

Results of cytotoxicity tests with exudate mononuclears in relation to duration of effusion

Although the number of observations in early effusion (of less than 3 months duration) was limited in each of the arthritis groups discerned attempts were made to relate the results of available cytotoxicity tests to the duration of effusion

In SLE and SLE like syndromes the shortest duration of effusion was one of 8 weeks and one of 5 months In both instances as in long standing effusion of this group positive tests were obtained (Table VI)

In psoriatic arthropathy five patients had effusion for a month or less Four of these showed positive cytotoxicity tests (Table VI) In long standing effusion all psoriasis patients showed a positive cytotoxicity test at least once

In RA as a whole positive cytotoxicity tests appeared somewhat more rarely in early than in long standing effusion (p = 0 18 Table VI) Six RA patients all sero positive like 8 with non RA arthritis (uro polyarthritis

Table VI Frequency of positive cytotoxicity tests with exudate mononuclears alone in relation to duration of effusion

Diagnosis	No of cases	Duration of effusion in weeks					
		<1	1—4	5—11	12—26	27—52	> 52
SLE and SLE like syndromes	9	—	—	1/1	1/1	2/2	5/5 (p = 0 004)
Psoriatic arthropathy	13	0/1	4/4	—	—	1/1	5/7 (p = 0 034)[1]
Rheumatoid arthritis	64	0/3	0/3	2/5	1/4	2/2	15/47 (p = 0 26)[1]
Miscellaneous arthritides[2]	22	0/5	0/3	0/2	0/1	0/1	1/10

[1] The frequency of positive tests in effusion of more than a year s duration was compared with that of 1/10

[2] Ankylosing spondylitis 11 uro polyarthritis 8 and oligo arthritis 3

and ankylosing spondylitis) had effusion of less than a month's duration All showed negative cytotoxicity tests (Table VI)

In long standing effusion of more than a year's duration the incidence of *positive cytotoxicity tests was found* to be higher in psoriatic arthropathy and in SLE and SLE like syndromes as compared with that in the group of miscellaneous arthritides ($p < 0.05$, Table VI) In RA on the other hand, there was only a tendency towards a higher incidence of positive tests ($p = 0.26$, Table VI)

When effusion had lasted for only a month or less the incidence of positive cytotoxicity tests in psoriatic arthropathy (4/5) seemed to be higher than that in RA (0/6), and also higher than that in non RA miscellaneous arthritides (0/8, $p < 0.03$, Table VI)

It should be added that in most cases of RA, exudation was fairly persistent once it had started In most of *the other arthritides and especially in* SLE and SLE like syndromes exudation tended to be more intermittent

SUMMARY Available observations showed that in psoriatic arthropathy a positive cytotoxicity test seemed to be an early phenomenon during the course of exudation

In RA positive cytotoxicity tests tended to be even more rare in early than in long standing effusion

CHAPTER 3

Cytotoxicity tests with cultures of human kidney as target

As a preliminary approach to the question of the organotypic specificity of the cytotoxicity of the exudate mononuclears, some tests were made with kidney cells as target. Kidney (human fetal) was selected as an epithelial organ which generally showed a fairly good outgrowth when cultured by the tissue culture technique used. Small pieces of kidney cortex were placed on the glass bottom of LEIGHTON tubes, whereafter the same tissue culture medium as that used for initiation of the fibroblast cultures was added. The medium was changed regularly and always as a matter of routine after 48 hours. Cultures of varying ages (4 to 18 days) were used.

In about half the number of tests kidney and fibroblast cultures of the same age were simultaneously challenged with exudate mononuclears. Owing to scarcity of exudate mononuclears a few tests were made with kidney cultures alone as target.

In (sero positive) RA (no nephropathy) the kidney cultures were found to be damaged as often as the fibroblast cultures (Table VII). In three cases the fibroblast cultures alone and in two the kidney cultures alone were damaged (Table VII).

In psoriatic arthropathy, on the other hand, the skin cultures but not the kidney cultures were damaged when exposed to exudate mononuclears (Table VII).

In two single cases of SLE-like syndrome (no nephropathy) similarly studied the result agreed with that obtained in psoriatic arthropathy: the fibroblast cultures alone were damaged.

In two cases of ankylosing spondylitis and two of uro-polyarthritis neither the kidney cultures nor the fibroblast cultures were damaged.

When in a few instances the cytotoxicity tests with kidney cells as targets were repeated, the results were reproduced.

In the tests with kidney cultures those explants were selected and photographed which contained only a few fibroblast-like cells. In one case of RA and one of psoriatic arthropathy it was found that at some of the other explants containing more fibroblast-like cells the exudate mononuclears had clustered around these but not around the epithelial cells (Fig. 4).

SUMMARY. Cultures of human kidney and those of (human) fibroblasts



Day 0



Day 1 (score on day 2 = 0)



Day 0



Day 1 (s ore = + +)



Conta tual agglut nat on
on f broblast l ke cell
of k dney culture (day 1)

F g 4 S m ltaneo s challenge of 7 days cultures of k dney cells and f broblasts ith exudate m ononuclears in a case of psor at c arthropathy

Table VII Results of simultaneous challenge of primary cultures of fetal human kidney and those of fetal human skin fibroblasts *with exudate mononuclears alone No trypsin* treatment The mean age of the two types of cultures was 12 days Approximately half the number of tests in each group was made with cultures of the same age and source The cytotoxicity score is given

Diagnosis	No of cases	Type of target cells	
		Kidney	Skin fibroblast
Psoriatic arthropathy	6	0	+ +
	1	0	N D [1]
	Total scored + +	0/7	6/6
RA with nodules	3	+ +	+ +
	2	+ +	0
	1	0	+ +
	1	0	N D [2]
	Total scored + +	5/7	4/6
RA without nodules	1	+ +	+ +
	2	0	+ +
	1	0	0
	Total scored + +	1/4	3/4
Sero poly arthritis	2	0	0
Ankylosing spondylitis	1	0	0
	1	0	N D [2]
SLE like syndrome	[illegible]	0	+ +

[1] Not done a previously made test had yielded a cytotoxicity score of + +
[2] Not done

were simultaneously challenged with exudate mononuclears

In RA (10 cases) the kidney cultures were damaged as often as the fibroblast cultures

In psoriatic arthropathy (6 cases) fibroblast cultures alone were damaged Similarly in two single cases of SLE like syndromes the fibroblast cultures alone not those of kidney cells, were damaged

Cytotoxicity tests with blood mononuclears

Mononuclears were separated from heparinized venous blood in the same way as that used for separation of exudate mononuclears, except that the bulk of erythrocytes was allowed to sediment in 1 2 per cent dextran (Macrodex, Pharmacia Sweden) for 40—50 min at room temperature As in the tests with exudate mononuclears an amount of 2×10^6 mononuclears per ml and test was used

Cytotoxicity tests with blood mononuclears which were made exclusively in cases of psoriatic arthropathy, RA and SLE and SLE like syndromes almost consistently yielded a negative result whether exudate mononuclears had elicited a cytotoxic effect or not (Table VIII) A positive test with blood mononuclears was obtained only in two cases one of RA (negative test with exudate mononuclears) and one of psoriatic arthropathy (positive tests with exudate mononuclears twice) The tests with blood mononuclears *and those with exudate mononuclears* were made on different occasions

Admixture with erythrocytes did not seem to influence the test results In a few (negative) tests most of the contaminating erythrocytes had been lysed by hypotone (0 35 per cent) saline according to a procedure described by JANOWSKI ROSENAU and MOON (1964) In some of the negative tests the suspension of blood cells had been pre treated with hyaluronidase

Table VIII Frequency of positive cytotoxicity tests with blood mononuclears alone For comparison the results of tests made with exudate mononuclears alone are included

Diagnosis	No of cases	Exudate mono nuclears	Blood mono nuclears
SLE or SLE	5	5/5	0/5
like syndromes	2	—[1]	0/2
Psoriatic arthropathy	9	9/9	1/9
Rheumatoid	3	3/3	0/3
arthritis	4	0/4	1/4
	2	—[1]	0/2
Total	25	17/21	2/25

[1] Tests not made

SUMMARY Cytotoxicity tests made with blood mononuclears instead of with exudate mononuclears were performed on 25 patients (7 with SLE or SLE like syndromes 9 with psoriatic arthropathy and 9 with RA) In 17 out of these 25, previously made tests with exudate mononuclears had turned out positive All tests with blood mononuclears except two (one case of psoriatic arthropathy and one of RA) turned out negative

CHAPTER 5

Discussion

Several methods have been developed for the quantitation of the cytotoxicity of lymphoid cells (see HOLM 1967) Such methods involve the use of certain ratios lymphoid cells/target cells as well as various techniques for the determination of the number of target cells damaged

Monolayer technique comparable to that used in the present investigation has been found useful in several studies (GOVAERTS 1960 KOPROWSKI and FERNANDES 1962 BERG and KALLEN 1963 1964 BJÖRKLUND 1964 HIRSCHHORN FIRSCHEIN and BACH 1965 KAKULAS 1966 and others) Related to the present technique is also the plaque technique (monolayer) used by MÖLLER and MÖLLER (1965) and MÖLLER BECKMAN and LUNDGREN (1966) In the latter technique however the ratio lymphoid cells/target cells is presumably subject to less variation than that in ordinary monolayer technique

A simple non quantitative monolayer technique was used in the present study which was designed as an approach to the question of whether exudate mononuclears can exert a cytotoxic effect on cultured fibroblasts in arthritis The intention was that if clear cut results were obtained and especially if distinct differences appeared between different form of arthritis the results would serve as a basis for further studies preferably performed by quantitative techniques

It may be mentioned that even under strictly standardized experimental conditions marked intra individual variations can appear in the cytotoxicity of lymphoid cells (HOLM and PERLMANN 1967) presumably because of the high degree of sensitivity of the method used (Cr^{51} release)

The present test system presumably ensured that essentially strong effects alone were revealed This refers to the capacity of the mononuclears to detach the fibroblasts from the glass Studies on the supernatant of cultures suggested that this effect would not necessarily result in the killing of all loosened fibroblasts

The fibroblasts were cultured directly on glass whereby cell damage induced by enzymatic tissue disintegration was avoided (EDWARDS and FOGH 1959 LEVINSON and GREEN 1965)

Since the first cytotoxicity tests made with exudate mononuclears yielded obvious differences between different forms of arthritis the procedure initially used (see Materials and methods) was retained

When fibroblast cultures of different sources and ages were simultaneously

General Summary

The present study of synovial fluid in various arthropathies deals with two phenomena one the suppression of the C′ activity, the other the occurrence of mononuclear cells with a capacity to elicit a cytotoxic effect on tissue cultured human fibroblasts Although the cytotoxic reaction elicited by exudate mononuclears had certain features in common with that elicited by immune lymphoid cells in various experimental conditions it is an open question whether the cytotoxicity of exudate mononuclears is analogous to that of immune lymphoid cells

In order to provide a survey an attempt was made to summarize the synovial fluid findings in Table I For comparison the conventional serum tests for RFs and ANFs were included

Thus totally four reactivities were considered the RF and ANF activity of serum the activation of the C′ system as determined by the C′ activity of synovial fluid and the occurrence in this fluid of mononuclears with an *in vitro* cytotoxic effect

For the sake of convenience various non RA sero negative arthropathies such as ankylosing spondylitis uro polyarthritis osteoarthrosis lesions of the menisci etc were treated together as one group of "miscellaneous arthropathies" included in this group were also cases of oligo arthritis

As shown in Table I different patterns could to some extent be discerned in different types of arthritis though various degrees of overlapping were evident too

In the heterologous group of *non RA sero negative "miscellaneous arthropathies"* all patients showed a non suppressed synovial fluid C′ activity considered to be essentially normal In this group only one patient (with ankylosing spondylitis) out of 27 showed a positive cytotoxicity test

In *psoriatic arthropathy* the outstanding result (Table I) was the occurrence of exudate mononuclears with an *in vitro* cytotoxic capacity, the other reactivities being present in a low frequency or entirely absent A suppressed synovial fluid C′ activity appeared in some patients where arthritis did not involve the terminal inter phalangeal joints

The *in vitro* cytotoxicity of exudate mononuclears in this group was directed against fibroblasts but not against kidney cells Positive cytotoxicity tests seemed to appear early in

Table I Summary of synovial fluid findings For comparison the conventional serum tests for RFs and ANFs are included — A simple score is used where

0 = an incidence of about 0 per cent
(+) = an incidence of about 10—15 per cent
+ = an incidence of about 25 per cent
+ + = an incidence of about 50 per cent
etc

Diagnosis	Serum findings		Synovial fluid findings			
	Positive ANF test	Positive RF test	Suppressed C' activity[1] Duration of effusion		Cytotoxic exudate mononuclears Duration of effusion	
			< 3 months	> 3 months	< 3 months	> 3 months
Psoriatic arthropathy	+	0	(+)	+	+ + +	+ + +
SLE and SLE like syndromes	+ + + +	+ + +	+ + + +	+ + + +	?	+ + + +
RA with nodules	+ +	+ + + +	+ + + +	+ + + +	?	+ +
RA without nodules	+	+ + +	+ +	+ +	(+)	+
Total RA	+	+ + +	+ +	+ +	(+)	+
Miscellaneous arthropathies[2]	?	0	0	0	0	(+)

[1] At the 5 per cent level
[2] Predominantly ankylosing spondylitis uro polyarthritis osteoarthrosis (only a few cytotoxicity tests) lesions of the menisci (no cytotoxicity tests) and juvenile oligo arthritis (only a few cytotoxicity tests)

this group of patients 4 out of 5 with effusion of less than a month s duration showed positive tests

Tests with blood mononuclears turned out negative in 8 out of 9 psoriasis patients

In the group of *SLE and SLE like syndromes* all four reactivities occurred in a fairly high frequency (Table 1) The patients in this group showed extremely suppressed synovial fluid C' activities and all (totally 9) showed positive cytotoxicity tests with exudate mononuclears whereas tests with blood mononuclears turned out negative

A suppressed synovial fluid C' activity seemed to be an early phenomenon in this group of patients

The organotypic specificity of the positive cytotoxicity tests however as

well as the occurrence of such tests in instances of early effusion, remain to be determined. So far only two cytotoxicity tests were made with kidney cells as target: both turned out negative.

In *rheumatoid arthritis* the essential finding was the presence of a highly significant degree of negative correlation between the synovial fluid C′ activity and the RF titre. This was true of each of the three RF titres determined: the SSC titre of (whole) serum and the latex titre (euglobulin fraction) of serum and synovial fluid.

The inverse relationship between the synovial fluid C′ activity and the RF titres was most clearly manifest in the group of RA with subcutaneous nodules. In this group the synovial fluid C′ activity was highly significantly lower and the RF titres of serum significantly higher as compared with corresponding parameters in RA patients without nodules. The RF titre of synovial fluid (by the latex test), however, was similar in the two groups compared.

The results suggested that a depressed synovial fluid C′ activity in different joints of the same patient was a more consistent phenomenon when the SSC titre of serum was markedly elevated. At intermediate SSC titres the intra-individual variance like the inter-individual one was considerable.

The results suggested that in RA a suppression of the synovial fluid C′ activity (probable at the 5 per cent level) is a late phenomenon during the course of exudation.

Positive cytotoxicity tests in RA appeared in a fairly low frequency of about 30 per cent. Most positive tests appeared in patients with subcutaneous nodules.

In early effusion positive cytotoxicity tests tended to appear even more rarely than in long-standing.

In this group of patients the cytotoxicity of exudate mononuclears was directed against kidney cells as well as against fibroblasts.

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	4 00	17	10 0	0 51	199	6	0 01
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Mean l of cases	4 31	1	1 3		M 1	31	4 9
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Appendix Table II RA WITH NODULES 27 adult patients Laboratory and clinical data

Total prot of synovial fluid g per 100 ml	C' units per ml		C'_{SF} C_S	C activity of synovial fluid[2]	ESR[3]	SSC titre (serum)[4]	Latex aggl titre[5]	Duration of the disease (years)
	synov fluid (C_{SF})	serum (C_S)[1]						
5 10	1143	1734 +	0 66	129	69	512xx	80/40	7
3 33	262	1208 –	0 22	66	61	64	40/<10	13
3 70	214	1251 –	0 17	46	31	16xx	10/<10	7
4 00	167	1250 +	0 13	39	48	32	40/20	4
5 73	244	1389	0 18	31	96	<16xx	80/80	1
5 70	256	1452 –	0 17	30	65	32xx	40/40	8
4 44	155	1299 +	0 12	27	49	64	80/40	10
3 22	97	1173 +	0 09	26	38	512	20/20	20
5 78	115	1068 +	0 11	20[6]	55	256	160/160	10
4 16	77	971 +	0 08	19[6]	123	1024	≥320/160	7
4 80	95	1046 –	0 09	[illegible]	55	512	40/10	12
4 44	72	1170 –	0 06	14	62	1024	160/80	7
3 78	65	1322 +	0 05	13	52	512	≥320/160	10
4 11	32	508 –	0 05	[illegible]	45	256	160/80	7

	4 59	64	990	0 06	*13*	*73*	< 16xx	40/160	1 5
	2 69	32	1107 +	0 03	11	37	512x	10/40	9
	4 78	12	700 +	0 05	*11*	34	64	—/20	31
	5 89	64	1000 +	0 06	11	125	1024	160/80	1
	4 05	32	851 −	*0 04*	9	24	64x	160/—	11
	5 56	59	1282 −	0 05	8	59	128xx	80/>320	17
	5 92	46	1010	0 05	*8*	*76*	128	80/80	9
	5 24	40	980	0 04	8	55	128	80/160	10
	5 10	31	909 +	*0 04*	7	*33*	64x	160/160	19
	4 56	32	952 +	0 03	7	56	256xx	>320/20	1
	4 70	32	1070	0 03	7	27	< 16 x	80/40	16
	4 50	32	1100 −	0 03	6	44	129	160/80	12
	5 23	32	1106	0 03	6	35	32	160/100	6
Mean	4 63	130	1107	0 10	Median 13	60	—	—	8 5

[1] + and − indicate the results of a test for ANI s in serum

[2] Figures to the left represent *effusion for* less than 3 months (= early effusion)

Italicized figures represent patients receiving corticosteroids *per os* or ACTH

x = gold treatment at least once during the 4th or the 5th year preceding examination

xx = gold treatment at least once during the *three years preceding* examination

[3] Titre of serum/titre of synovial fluid

[4] Concomitant psoriasis

Appendix Table III SSC LOS RA 49 adult patients Laboratory and clinical data

Total prot of synovial fluid g per 100 ml	C units per ml: synov fluid (C_{SF})	C units per ml: serum (C'_S)[1]	C'_{SF} C'_S	C activity of synovial fluid[2]	ESR[3]	SSC titre (serum)[4]	Latex aggl titre[5]	Duration of the disease (years)
3 58	414	1214 –	0 34	95	16	<16	<10/<10	0
5 00	185	1042 +	0 47	94	41	<16xx	<10/<10	12
6 01	619	1087 –	0 57	94	87	<16xx	20/20	7
2 89	410	1500 –	0 27	93	101	16	10/<10	1 5
3 78	421	1269 –	0 33	97	27	128	40/<10	0 6
4 89	400	1089	0 37	76	28	128	160/160	1 8
3 38	299	1250	0 24	71	29	<16	<10/10	8
4 18	340	1191 –	0 28	67	52	32	10/20	1 2
5 89	426	1099	0 39	66	39	64x	40/10	5
6 22	600	1587 –	0 38	61	40	32	80/>320	8
5 42	403	1277	0 32	59	86	64x	80/—	0
5 00	414	1613 +	0 26	52	52	256	160/160	16
5 70	388	1282	0 30	52	61	128	160/80	1
5 07	312	1265 –	0 25	49	63	<16	10/20	9
3 07	287	1563 +	0 18	49	14	1024	80/160	30
4 50	220	1055	0 21	47	64	32	160/80	9
4 65	220	1025	0 21	45	72	16	160/160	10
3 88	141	810	0 17	44	11	64	10/160	5
5 24	261	1220	0 22	42	70	32	80/160	5
4 07	278	1623 +	0 17	42	55	128	80/20	7
4 78	268	1334 +	0 20	42	34	64	40/40	1 2
5 67	341	1515 –	0 23	41	110	512	80/80	0 9
5 85	345	1497	0 23	39	90	128	160/40	6

	5.88	377	1724 –	0.22	37	60	128	80/80	7
	5.00	215	1250	0.17	34	61	64	40/40	4
	5.33	200	1136	0.18	34	50	128	20/40	8
	4.71	196	1250 –	0.16	[illegible]	95	512	160/160	8
	4.87	163	1096 –	0.15	31	54	64	40/20	0 2
	4.71	192	1326 –	0.14	30	70	256	> 160/40	2 4
	6.15	221	1250 –	0.18	29	29	128	40/160	16
	5.87	190	1131 –	0.17	23	113	< 16*	> 320/—	[illegible]
	6.67	252	1310 +	0.19	28	60	16**	10/20	6
	6.50	350	1389 –	0.18	28	89	< 16	40/160	7
	4.70	140	1905 +	0.12	26	50	32**	40/160	3 3
	4.05	158	1130	0.10	25	30	128*	80/80	1 5
	5.11	156	1391	0.11	22	23	64	160/80	7
	5.04	150	1214	0.10	20	49	32*	40/160	14
	5.44	124	1250 –	0.10	19	53	128	80/160	23
	4.15	41	619 –	0.07	[illegible]	30	512	40/80	11
	5.14	104	1938 +	0.09	16	47	512	80/80	4
	5.11	59	1061 –	0.09	16	71	256	80/160	13
	4.[illegible]	60	935 –	0.07	16	49	512*	> 320/160	6
	4.[illegible]	50	905	0.06	13	50	64	80/160	10
	4.55	72	1214 –	0.06	13	41	128**	80/160	8
	5.[illegible]3	70	1220	0.06	11	90	256	80/80	0 8
	5.00	57	1200 +	0.05	10	[illegible]	64*	80/160	7
	5.78	54	868 +	0.06	10	70	128	160/160	1 4
	3.70	95	1016 –	0.03	9	23	< 16*	< 10/40	9
	5.44	95	1333 –	0.03	5	112	64*	80/80	11
Mean	4.94	213	1230	0.19	Median 34	57			7.0

*, ** and * see Appendix Table II

Appendix Table IV SSC NEG RA 12 adult patients Laboratory and clinical data

	Total prot of synovial fluid (g per 100 ml)	C' units per ml: synov fluid (C'_{SF})	C' units per ml: serum (C'_S)[1]	C'_{SF} / C'_S	C' activity of synovial fluid[2]	ESR[3]	SSC titre (serum)[4]	Latex aggl titre[5]	Duration of the disease (years)
	1 12	412	1111 –	0 40	97	95	< 16 –*	< 10/ < 10	8
	5 49	577	1111 –	0 52	95	75	< 16 –	< 10/ < 10	13
	4 44	538	1214	0 44	90	51	< 16 –	< 10/ < 10	10
	5 11	605	1306 –	0 44	86	22	< 16	< 10/ < 10	0 5
	2 89	277	1197 –	0 23	80	17	< 16	10/ < 10	12
	7 51	209	1000 –	0 21	60	35	16 –	40/ > 320	3
	7 39	263	1407 –	0 19	56	14	< 16 –	80/80	14
	5 33	357	1250 +	0 29	53	50	< 16 –	< 10/ < 10	1 9
	5 22	187	808 –	0 23	44	20	< 16	< 10/ < 10	13
	5 20	303	1563 –	0 10	36	115	< 16 –	20/ < 10	7
	6 02	147	1185 –	0 12	20	99	< 16 –	40/40	14
	4 42	74	1092 –	0 07	16	37	< 16 – xx	< 10/ < 10	3
Mean	4 67	330	1192	0 30	Median 58	52	—	—	9 3

[1] [2] [3] [4] and [5] see Appendix Table II   * – indicates a negative SSC test at re examination 1—3 years later

Appendix Table V JUVENILE ARTHRITIDES 26 patients Laboratory and clinical data

	Total prot of synovial fluid g per 100 ml	C' units per ml: synov fluid (C'_{SF})	C' units per ml: serum (C'_S)[1]	C'_{SF} / C'_S	C' activity of synovial fluid[2]	ESR[3]	SSC titre (serum)[4]	Latex aggl titre[5]	Duration of the disease (years)[6]
SSC pos RA									
	3 12	250	929*	0 27	87	65	16xx	< 10/ < 10	5_{8}
	4 67	256	1037 –	0 25	54	47	32	80/80	$0\ 5_{15}$
	4 24	274	1559	0 18	42	55	256	80/160	$0\ 8_{11}$
	5 20	227	1401	0 16	31	72	512xx	160/80	6_{14}
	5 10	162	1250	0 13	25	17	512	160/160	$2\ 3_{12}$
	4 70	137	1191 –	0 11	23	55	1024	160/80	2_{10}
	3 33	96	1000	0 10	19	59	32	160/80	$1\ 1_{12}$
Mean	4 65	200	1195	0 17	Median 31	57	—	—	$3\ 0_{12}$

SSG eg RA									
	5 4	91	118	0 66	120	1	<10 – x	<10 <10	6
	4 36	69	1 08	0 4	103	2	<10	<10 <10	13_2
	4 0	4 0	11 6	0 1	11	7	<10 –	<10/<10	1_7
	33	3 6	13 –	0 36	68	17	<10 –	<10/10	1_5
	4	95	69	0 1	9		<10	<10/<10	0_9
M	1 80	47	1110	0 40 Me lia	91	28	—	—	$0 0_{46}$
Ol go artl r tis									
	$3 60_4$	89	1333	0 36	12		<10	<10/<10	$1 1_0$
	$2 98_4$	306	10 0	0 36	1 1	7	<10 –	<10/<10	4_{15}
	$4 90_3$	9 8	10 3	0 6	114	3	<10	<10/<1	1_2
	$3 40_2$	311	893	0 39	11	3	<10	<10/<10	$0 3_{15}$
	4 90	77	1016	0	112	1	<10 –	<10/<10	$0 6_9$
	37_2	664	113	0 6	101	3	<10	<10/<1	0_8
	$4 6_2$	3	1 0	0 38	103	8	<1 –	<1/<10	7_3
	$1 68_3$	370	881	0 4	0		<10	<10/<10	1_{11}
Men	$4 31_{23}$	517	1147	0 1 Me li	11	18	—	—	$3 8_3$
A kyl b 1 o lyl tis									
	4 30	18	1 7	0 48	11	0	<10	<1 /<10	8_9
	1 42	3	11	0 3	89	11	<10	<10/<10	11
	0 67	2 6	8 0	0 3	4	41	<10	<1 /<10	7_{12}
M a	13	4	1131	0 10 M 1 a	88	1	—	—	8_{11}
Alyl f lj l a ltr s									
	5 30	7 4	1 66	0 6	111	60	<10	<1 /<10	1_5
l orl s l n e c l is lar l itis									
	11		1161	0 1	6	1	<10	<10 <10	10
S 1 l									
		98	1 6	1	7	1 7	<10	<1 <1	$0 01_{12}$

a l s A l l l x Fal ll
l lex l l s ag l s t f j l s l s l l l a n g ll SS t t l r x l ll l—l y rs l t r
L l a yl l als r f l l s ll y s y l l x l l l s ll f j l ts l l e l

Appendix Table VI. Systemic lupus erythematosus (SLE) and SLE-like syndromes. 10 adult patients. Clinical and laboratory data

Age and sex	LE cell tests	Cutan. LE	Score[a]	Total prot. of synovial fluid g per 100 ml	C' units per ml		C'_{SF}/C'_S	C' activity of synovial fluid[c]	ESR[d]	SSC titre (serum)[e]	Latex agglut. titre[f]	Duration of joint symptoms (years)
					synov. fluid (C'_{SF})	serum (C'_S)[b]						
38 F	+	+	11	4.36	99	1104+	0.09	19	57	<16	<10/<10	1.2
22 F	+	+	8	4.56	<32	516+	0.06	13	30	512xx	>320/160	4
55 F	+	–	10	4.78	41	1017+	0.04	9	65	<16	40/40	2.5
61 F	+	–	7	5.41	<32	414+	0.08	14	120	256	160/40	7
40 F	+	–	9	4.96	<32	996+	0.03	7	87	32xx	10/<10	6
13 M	+	–	6	2.79	<32	1316+	0.02	9	93	512	80/80	10
22 F	–	+?	10	4.45	132	1471+	0.09	20	102	1024	80/50	6
57 F	–	–	10	3.78	<32	199+	0.17	45	29	16	80/>320	11
30 F	–	–	9	4.65	71	1166+	0.06	13	21	<16xx	<10/20	1.3
55 F	–	–	7	5.67	193	1057–	0.17	30	120	32	80/>320	0.3

a, b, c, d, e see Appendix Table II

f According to Leonhardt (1964)

Appendix Table VII PSORIATIC ARTHROPATHY 14 patients 13 adult and one juvenile (the fourth case) all with negative tests for RFs in serum and synovial fluid Laboratory and clinical data

Total prot of synovial fluid (g per 100 ml)	C units per ml		C_{SF}/C_S	C activity of synovial fluid	ESR	Duration of arthritis (years)
	synov fluid (C_{SF})	serum (C_S)				
4.80[1,2]	1128	1849 –[5]	0.61	127	112	8
4.08[1]	610	1388 +	0.44	108	55	5
4.44[1]	455	996	0.46	104	13	[illegible]
4.50[1]	591	1295 +	0.45	100	32	[illegible]
4.96[1]	514	1163	0.48	97	68	[illegible]
4.81[1,2]	612	1362 –	0.45	94	29	8
3.56[1,2]	444	1418 –	0.31	87	25	3
4.55[1,3]	407	1149 +	0.35	77	111	[illegible]
3.38	335	1292 –	0.26	77	43	2
6.40[1]	865	1997 –	0.46	72	101	1
3.67[4]	327	962	0.34	97	39	1
4.11[4]	204	1244 –	0.16	30	125	27
6.00[4]	295	1325 –	0.22	37	25	7
4.79[4]	<32	1292 +	0.02	5	64	13
Mean 4.57	493	1370	0.37 Median	90	61	7.0

[1] Involvement of the distal interphalangeal joints
[2] Sacro-iliac arthritis
Symphysitis—no sacro-iliac arthritis
[4] Squaring of the vertebrae—no sacro-iliac arthritis
[5] ANF test, early effusion and treatment with corticosteroids per os or with ACTH are marked as in Appendix Table II

Appendix Table VIII The C activity of different synovial fluids simultaneously aspirated from different joints of the same PA patient

Joint 1	Joint 2	Joint 1	Joint 2	Joint 1	Joint 2	Joint 1	Joint 2
120	90	52	61	27	21	20	10
97	44	72	36	31	69	16	75
71	77	2	24	71	26	13	9
58	58	67	67	71	20	10	12
67	24	37	57	25	[illegible]	9	8
59	27	39	70	27	2[illegible]		

[1] Generally the right knee joint
[2] Generally the left knee joint

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TO THE OCCURRENCE OF VARIOUS PLASMA PROTEINS
IN THE DERMAL VESSEL WALLS

BY

OLLE LARSSON

UMEÅ 1967

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STUDIES OF SMALL VESSELS IN PATIENTS WITH DIABETES

A CLINICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF DIABETIC AND NON-DIABETIC SUBJECTS WITH SPECIAL REFERENCE TO THE OCCURRENCE OF VARIOUS PLASMA PROTEINS IN THE DERMAL VESSEL WALLS

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OLLE LARSSON

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CONTENTS

INTRODUCTION

In recent years many studies have been devoted to the distribution and the appearance of changes in small blood vessel walls in diabetes mellitus as seen under the light and electron microscopes Several histochemical, chemical, and immunohistochemical investigations have been carried out in an attempt to elucidate their nature

The present investigation was initiated by observations made by Odin & Tornblom (1959) in cooperation with Muller Eberhard (1959) Using an immunoelectrophoretic technique, they were able to demonstrate that mechanically isolated, washed hyaline glomeruli from diabetic kidneys absorbed anti human serum protein sera whilst similarly treated glomeruli from apparently normal non diabetic kidneys did not do so to the same extent Furthermore, Freedman *et al* (1960) and others, who used the fluorescent antibody technique, were able to show that human γ globulin was localized in the region of the vascular glomerular basement membrane and in other minor vessels of the diabetic kidney

Many investigators have shown that small blood vessels in apparently normal skin from patients with diabetes become involved in the diabetic »microangiopathy» at an early stage of the disease and in a high proportion

As it is easy to obtain large series of skin biopsies, these observations presented the possibility of studying the possible localization of γ globulin (and other plasma proteins) in small blood vessel walls more systematically in biopsy material from patients with diabetes mellitus and from non-diabetic individuals

The present investigation represents an extension of two preceding reports (Larsson & Melin, 1964, Larsson, 1966) It comprises the following

1 An histological study of the PAS-reaction of small dermal blood vessels by means of light microscopy in patients with diabetes and in matched non-diabetic individuals

2 An immunohistochemical study of the occurrence of γG globulin in the walls of small dermal blood vessels in patients with diabetes mellitus and in matched non diabetic individuals

3 An attempt to detect any clinical features of the diabetic state which may influence the periodic acid Schiff (PAS) reaction and the localization of γG globulin in the vessel walls

4 An immunohistochemical study of the occurrence of some other plasma proteins in the walls of small dermal vessels in patients with diabetes mellitus and in matched non diabetic individuals

The immunohistochemical studies were performed by means of the fluorescent antibody technique

The object of these four parts of the study was to obtain a more thorough knowledge of the frequency and nature of the small blood vessel involvement in patients with diabetes mellitus

5 In various other diseases, *e g* glomerulonephritis, systemic lupus erythematosus (SLE) and rheumatoid arthritis (Douglas, 1965, Rodman *et al* 1967), as well as in experimental hypertensive vascular disease in rats (*cf* Giese, 1966) γ-globulin, complement and/or other plasma proteins have been demonstrated in blood vessel walls During the course of this investigation skin biopsies from a few patients with some diseases other than diabetes mellitus were therefore studied in order to obtain a basis for comparison with the results in patients with diabetes and in apparently healthy individuals

CHAPTER I

BRIEF SURVEY OF THE LITERATURE ON MORPHOLOGICAL CHANGES IN SMALL BLOOD VESSELS IN PATIENTS WITH DIABETES MELLITUS

Distribution in the body

The earlier literature dealing with changes in small blood vessels in the kidney and the eye in diabetes mellitus has been reviewed by Ashton (1958) In recent years many investigators have described basically similar, probably non arteriosclerotic changes in small blood vessels in diabetes — »diabetic microangiopathy» — in various organs Thus small vessel involvement has been found in the peripheral and central nervous systems (*cf* Fagerberg 1959 and Reske Nielsen *et al* 1965) in intramural vessels of the heart (Blumenthal *et al* 1960) in vasa vasorum (*cf* Angervall *et al* 1966) in skeletal muscles (*cf* Bencosme *et al* 1966 Siperstein *et al* 1966) in synovial membranes (Aagenaes & Hagensen 1959) in the digestive tract (*cf* Angervall & Save Soderbergh 1966) and in exocrine and endocrine glands (*cf* Berns *et al* 1964 Funk 1965 Angervall & Save Soderbergh 1966) Reports on dermal blood vessel changes have been listed in Table 1

Many problems in connection with the vascular changes in diabetes have recently been discussed by Siperstein *et al* (1964) Berkman & Rifkin (1966) Stary (1966) and Warren *et al* (1966)

Light microscopic changes

A thickening of the peripheral vessel wall has been considered to be a characteristic finding in diabetes (*cf* Siperstein *et al* 1964) This thickening is most clearly visible in capillaries but is also seen in arterioles and venules The PAS staining (McManus, 1946) has been widely used to study the changes in small blood vessels The lesions in small dermal blood vessels in diabetes was first described in more detail by Aagenaes & Moe (1961) Their findings have to the greater part been confirmed by others (Table 1)

The PAS positive thickening can either be seen as a homogenous thickening of the entire periendothelial vessel wall or as a thickened and sometimes split up basement membrane Occasionally pericytes are seen encased in the basement membrane The changes are further characterized by an uneven, segmental distribution (Pedersen & Olsen 1962) Seemingly normal and conspiciously altered vessels may be observed in the same specimen

In some studies the wall thickness has been arbitrarily graded (Banson & Lacy 1964 Angervall *et al* 1965 Pieri *et al* 1965 Save Soderbergh *et al*, 1967) sometimes based on micrometer

TABLE 1 *Microangiopathy in dermal vessels in diabetics*

	Biopsy / Necropsy / Amputation	Site	Light Microscopy	Electron Microscopy	Thickened Vessel Wall	Endothelial Proliferation	Positive Diabetics (%)	Positive Non diabetics	Number Investigated Diabetics	Reference		
1	A	Leg	+		+	+	63 %	5 %	51	Goldenberg *et al*		1959
2	B/N	Thumb Toe	+	(+)	+	—	71 %	1/8	24	Aagenaes & Moe		1961
3	[illegible]	Fore arm	+		+	+	79 %	1/13	19	Handelsman *et al*		1962
4	B/A	Leg	+		+	+	63 %		20	Pedersen & Olsen		1962
5	[illegible]	Toe	+		+	—	50 %	17 %	40	Weber & Wicht		1962
[illegible]	N		+	+	+	—				Bloodworth		1963
7	[illegible]	Finger	+		+		60 %	0 %	10	Bojsen Møller *et al*		1963
[illegible]	B/N	Leg	+		+		82 %	0/5	11	Mehn		1964
9	N/A	Toe	+	(+)	+	—	68 %	23 %	18	Banson & Lacy		1964
10	B	Hand	+		+		91 %	14 %	24	Faerman *et al*	1954	1965
11	B	Fore-arm	+		+		++	+	44	Bercovici *et al*		1964
12	B		+		+		++	?	(27)	Garrachon *et al*		1965
13	B/A	Leg	+		+	+	90 %	6 %	52	Moore & Frew		1965
14	N	Leg	+		+	+	68 %	21 %	50	Funk		1965
15	B	Ear	+		+	—	100 %	3/15	38	Piers *et al*		1965
16	B	Foot	+	(+)	+	—	++	+	65	Angervall *et al*, Save Soderbergh *et al*	1965	1967
17	[illegible]	Finger	(+)	+	+/—		+/—	+/—	13	Friederici *et al*		1966
18	B	Fore arm	+	(+)	+	—	53 %	7 %	30	McMillan *et al*		1966
19	[illegible]	Finger	+	+	+	+	100 %	1/10	11	Durand & Durand		1966
20	[illegible]	Fore arm	(+)	+	+	—	++	+	31	Pardo *et al*		1966
21	[illegible]	Ear	(+)	+	+	+	++	+	35	Otto *et al*		1967
22	[illegible]	Fore arm		+	+	—	+/—	+/—	21	Yodaiken *et al*		1967

(+) indicates that the *main* results and conclusions of the study concerned are not based primarily on this particular investigation.

measurements of selected capillaries (Bercovici *et al* 1964)

Although as a rule thickened vessel walls have been a common finding in diabetic subjects these changes are by no means limited to the diabetic state, as emphasized by Bercovici *et al* (1964), »the presence or recent history of even minor skin complaints may drastically alter the light microscopic interpretation of microangiopathy in the skin»

Changes in endothelial cells, especially in arterioles and small arteries, are

considered by some to be a rather characteristic lesion in diabetes (*cf* Blumenthal *et al*, 1964) The lesion is described as ■ proliferation of the cells These are often enmeshed in fine PAS-positive fibrils It has been suggested that the lesion has an »immunogenic» basis It can occur in such diseases as chronic glomerulonephritis, systemic lupus erythematosus, rheumatoid arthritis and malignant lymphomas It has also been observed in homografts Likewise, Blumenthal *et al* (1964) found a similar lesion in rabbits immunized with bovine insulin The findings in diabetes have been confirmed by some and questioned by others (Table 1, Warren *et al*, 1966)

Other investigators have stressed that changes in the pericytes may be of etiologic importance for the development of the small blood vessel lesions in diabetes mellitus (*cf* Kimmelstiel 1966, Yodaiken *et al*, 1967, Säve Soderbergh *et al*, 1967) Others maintain that the connection of the function of the pericytes to the vascular changes in diabetes mellitus is still unsubstantiated (Oliveira 1966)

Ultrastructural changes

Ultrastructural studies have confirmed the presence of focal thickenings of capillary walls in patients with diabetes They have also confirmed that similar changes occur in non-diabetic individuals (Bloodworth, 1962, Friederici *et al*, 1966)

Under the electron microscope an uneven thickening and sometimes a splitting up of the periendothelial basement membrane can be seen together with an accumulation of basement membrane like material in the perivascular space In advanced lesions the accumulation of substances, probably lipoid- and protein rich has been noted The »basement membrane» observed under the light microscope probably consists of both the ultrastructurally defined periendothelial basement membrane and the seemingly amorphous material in the perivascular cuff of the dermal capillaries (Pardo *et al*, 1966, Otto *et al*, 1967)

Careful measurements of different parts of the basement membrane of dermal capillaries (Friederici *et al*, 1966 Pardo *et al*, 1966 Otto *et al*, 1967) and of the peripheral glomerular basement membrane (Østerby Hansen, 1965, Kimmelstiel *et al*, 1966) in sections from diabetic and non diabetic subjects have shown great variations in the basement membrane width in both groups with overlapping frequency distribution curves of the membrane thickness

In the skin at least the overlapping in wall thickness of small blood vessels in diabetic and non diabetic subjects, observed both under the light and electron microscopes makes it difficult to state with any certainty how early in the course of the diabetic disease the described vessel changes can be seen Several investigators have maintained that changes in small dermal vessels are an early manifestation of diabetes mellitus, and that they can be observed long before the disease becomes manifest (*cf* Camerini Davalos, 1965, Gar-

rachon *et al*, 1965, Faerman *et al*, 1965) It is clinically well known that the frequency of observed vascular lesions (*e g* retinopathy), increases in propotion to the duration of diabetes mellitus (*cf* Ashton, 1958) However, an increase in the small dermal blood vessel thickness in proportion to the duration of the disease was not found by some investigators (Pedersen & Olsen, 1962, Banson & Lacy, 1964, Friederici *et al*, 1966, Yodaiken *et al*, 1967) whilst others did find a connection between the vessel wall thickness and the duration of diabetes (Aagenaes & Moe, 1961, Weber & Wicht, 1962, Bercovici *et al*, 1964, Moore & Frew, 1965, Funk, 1965, McMillan *et al*, 1966 Otto *et al*, 1966, Säve Soderbergh *et al*, 1967) The disagreement may be due to the fact that the connection is difficult to show in a small series In the former group of studies referred to above, the number of diabetic subjects examined varied between 13 and 21, whilst in the latter group the number was from 24 to 65

Histochemistry

It has not been possible to demonstrate any significant qualitative differences in the composition of small blood vessels between diabetic and non diabetic subjects by histochemical methods Neither fibrinoid nor amyloid can as a rule be demonstrated in small vessel lesions in diabetes Exsudative lesions in the kidneys and severe arterial lesions in the gastro intestinal tract give staining reactions which indicate the presence of a mixture of protein and fat, »fibrinoid« (Lendrum, 1963, Angervall & Säve Soderbergh, 1966) Localized clumps of dark osmiophilic material, probably lipid, have been observed under the electron microscope in the basement membrane of muscle capillaries in diabetes (Bencosme *et al*, 1966) In this respect, the histochemical resemblance to findings in lipoglycoproteinosis may be noted (Falkmer *et al*, 1966) The amount of acid mucopolysacharides did not seem to increase in small vessels in diabetes The results of the colloidal iron reaction of Rinehardt and Abul Hai was negative (Goldenberg *et al* 1959)

Small vessel walls from diabetic and non diabetic subjects stain red when the PAS reaction is performed The reaction cannot be eliminated by pre treatment of the tissue with amylase In paraffin embedded sections, subsequently deparaffinized with fat solvents, this reaction is considered to be discriminating in detecting glycoproteins (*cf* Spiro, 1963)

Chemical analysis

Normal human glomerular basement membranes have been analyzed using different methods (Windrum *et al*, 1955, Robb Smith, 1957) A main constituent seems to be carbohydrate rich proteins with a high hydroxyproline content belonging to the reticulin collagen group of proteins These substances give PAS positive staining reactions Direct chemical analysis of the content of the normal peripheral capillary wall does not seem to have been performed (Meier 1964, Muir, 1964)

Similar antigenic determinants in the glomerular capillary basement membrane and in capillary basement membranes from other sources may indicate similarities in the chemical composition (Krakower & Greenspon, 1964, Cruickshank, 1964)

Odin & Törnblom (1959) carried out chemical analyses of mechanically isolated washed glomeruli from necropsy material Glomeruli with severe diabetic nephropathy were compared with those from apparently normal non diabetic kidneys No great chemical differences were found between the two categories of material Judging by the hydroxyproline content, about 1/3 of the proteins consisted of reticulin collagen like substances The presence of tryptophane indicated that non collagenous proteins were present By means of immunoelectrophoresis the diabetic glomeruli were found to contain almost all identifiable serum proteins Traces of gammaglobulin only were found in the non diabetic glomeruli

Also Lazarow & Speidel (1964) found that the principal part of the glomerular basement membrane consisted of collagen or collagen like substances both in diabetic and non-diabetic kidneys The results of amino acid analysis showed that probably no plasma proteins were present in the specimens As pointed out by Spiro (1964), the prolonged treatment of the glomeruli in alkali may have destroyed or eluted some proteins before the final analysis

Immunohistochemical investigations

The fluorescent antibody technique provides a fair possibility of identifying and histologically localizing individual or groups of antigenically closely related substances in different tissues

Using this method, Taft *et al* (1958) found traces of fluorescence indicating the presence of gammaglobulin in the glomerular basement membrane in a biopsy specimen from a patient with diabetes mellitus Freedman *et al* (1960, 1962) found gammaglobulin in diabetic kidneys not only in the glomerular basement membrane but also in the walls of other blood vessels in the kidney It was also possible to demonstrate complement with identical localization The immunofluorescence picture was very similar to that found in kidneys from patients with chronic glomerulonephritis and SLE These observations have been confirmed by others (Table 2) A survey of the localization of different plasma proteins in various vascular structures in diabetes mellitus is given in Table 3

The insulin binding capacity of the diabetic vascular lesions in the kidney was tested by Berns *et al* (1962) Fluorescein conjugated bovine insulin was used In diabetic nodular glomerulosclerosis 95 % of the 25 investigated kidneys showed positive fluorescence in the glomerular lesions Similar fluorescence was demonstrated in the PAS-positive fibrillar network in vessels with »proliferative» endothelial lesions, in the tubular basement membrane and cells and in Bowman's capsule This binding was noticed in 13 % of the dia-

TABLE 2 *Plasmaproteins demonstrated in vascular structures in diabetes mellitus by fluorescent antibody technique*

Source of the specimens	Necropsy, Biopsy or Amputation	Diabetes				Other diseases involving the specimens studied				Other specimens studied »Controls«				Reference	Reference Number
		Al	Fi	C	G	Al	Fi	C	G	Al	Fi	C	G		
Kidney	B				1/1				8/23					Taft *et al* 1958	I
»	B N				3/3				29/31				0/9	Freedman *et al* 1960	II
»	B				1/5				14/67					Mackay *et al* 1961	III
»	B N			1/1	1/1			12/12	12/12			0/6	0/6	Freedman *et al* 1962	IV
»	N			+)	14/41				9/42				0/5	Berns *et al* 1962	V
»	N				+/20									Moran *et al* 1962	VI
»	N	3/5	2/5	+)	6/6									Burkholder 1965	VII
»	N	0/23	13/23		0/23*)									Davies *et al* 1966	VIII
Retina	A, N	0/15			15/15	0/25			21/25	0/40			6/40	Coleman *et al* 1963	IX
Pancreas	N	0/12	5/16		19/23	0/3	0/2		8/14	0/9	2/12		3/7	Berns *et al* 1964	X
Placenta					11/11				5/7				0/5	Burstein *et al* 1963	XI
Leg	A				23/39				9/31					Blumenthal *et al* 1964, 1966	XII
Skin	B				6/6				2/2				0/2	Larsson & Melin	XIII
»	B	3/20	6/20	3/20	55/82					2/11	0/11	0/11	7/35	Larsson 1966	XIV

The figures indicate Number positive/Total number studied

Al Albumin.

Fi Fibrinogen

C Complement

G γ globulin (Anti human globulin serum was used by some)

) Binding of Guinea Pig complement *in vitro*

) Low density lipoproteins were demonstrated with identical localization and frequency as fibrinogen

COMMENTS TO TABLE II

Reference number	
I	Systemic lupus erythematosus 6/10 Glomerulonephritis 2/9
II	Systemic lupus erythematosus 8/8 Glomerulonephritis 8/8, Scleroderma 4/4 Amyloidosis 1/1 Rheumatoid arthritis 1/1 Uncertain diagnosis 1/1
III	Systemic lupus erythematosus 8/15 Glomerulonephritis 5/21, Toxicosis gravidarum 1/5
IV	Systemic lupus erythematosus 7/7 Glomerulonephritis 4/4
V	Systemic lupus erythematosus 2/3 Amyloidosis 2/7 Myelomatosis 4/5 Nephrosclerosis 1/15
IX	Glaucoma 21/25 (Positive fluorescence in trabecular meshwork)
X	The group »Other diseases involving the studied specimens« includes specimens from non-diabetic individuals with hyaline islets
XI	Rh incompatibility 5/7
XII	The group »Other diseases involving the studied specimens« includes Arteriosclerosis Mb Bürger Sarcoma Osteomyelitis Cold injuries
XIII	Systemic lupus erythematosus 2/2
XIV	Apparently healthy individuals (35)

betic kidneys without glomerular lesions The non diabetic kidneys investigated did not show any fluorescence The study was based on necropsy material and it was not possible to state whether insulin treatment was given to the diabetics or not

Similar investigations including organs other than the kidney have since been repeated by others (*cf* Table 4)

As can be seen in Table 4, insulin-binding has been demonstrated mostly, but not selectively, in tissues from diabetic subjects

It has been supposed (Berns *et al*, 1962, Blumenthal *et al*, 1966) that insulin is bound to the tissue by an antigen antibody reaction Anti insulin antibodies, localized *in vivo* in tissues are thought to bind the conjugated insulin Some support for this hypothesis has been given by observations that human γ globulin could be demonstrated in the same location where insulin was bound However, whereas 96 % of the diabetic glomerulosclerotic kidneys showed specific fluorescence when treated with conjugated insulin *in vitro*, localized human globulin could only be demonstrated with the immunofluorescent method in 48 % of the material Moreover, the fluorescence indicating the presence of human globulin was in most instances rather weak in the nodular lesions of the glomeruli whilst the fluorescence indicating bound insulin in these same lesions was strong (Berns *et al*, 1962) The presence of circulating anti insulin antibodies was only tested in a few of those patients from whom kidney sections were studied later on and the *in vitro* fixation of conjugated insulin was demonstrated In no case were such antibodies demonstrated (Farrant & Shedden, 1965)

Fluorescein conjugated anti insulin serum was used to study the localization of insulin *in vivo* in specimens from kidney and pancreas (Table 4) In the pancreas insulin was found in

TABLE 3 *Immunohistochemical localization of some plasma proteins in vascular and non vascular structures in various organs from diabetic subjects Reference numbers see Table 2*

A Localization in the kidney

Ref No	Non glomerular vessel walls				Glomerular capillary BM				Nodular lesions				Exsudative lesions			
	Al	Fi	C	G	Al	Fi	C	G	Al	Fi	C	G	Al	Fi	C	G
I								(+)								
II				+				+				+				
III								(+)								
IV			+	+			+	+			+	+				
V			+)	+			+)	+				(+)				
VI	?	?		+	?	?		?	—	—		—	+	+		+
VII	—	+	+)	+	+	+	+)	+	—	—	—)	(+)	—	—	+)	+
VIII	—	+		—	—	+		—	—	+		—	—	+		—)

B Localization in other organs than the kidney

	Retina		Islets of Langerhans		Placenta		Amputated legs	Skin biopsy	
	Vessel BM	Micro aneurysms	Vessel wall	Islet tissue	Vessel wall	Other BM	Vessel BM	Small vessel BM	
Al	—	—	—	—					+
Fi			+	—					+
C									+
G	+	+	+	+	+	+	+	+	+
Ref No	(IX)		(X)		(X)		(XII)	(XIII)	(XIX)

Al = Albumin Fi = Fibrinogen C = Complement fractions G = γ globulin) = *in vitro* binding of guinea pig complement) = Low density lipoproteins were demonstrated in the same localization and frequency as fibrinogen (+) = Weak specific fluorescence ? = Possible localization not stated

TABLE 4 *In vitro binding of fluorescein conjugated insulin to blood vessel walls and the demonstration of in vivo localized insulin by aid of fluorescein conjugated anti insulin serum*

Source of the specimens	Diabetes				Other		Reference	Reference Number
	Necropsy or Amputation	Insulin	Anti insulin	Not treated with insulin	Insulin	Anti insulin		
Kidney	N	27/41	50—74 %	?	0/56	0/15	Berns *et al* (1962), Blumenthal *et al* (1964)	V
»	N	+		?			Coleman *et al* (1962)	XV
»	N	28/32		4/9	3/40		Farrant & Sheddon (1965)	XVI
»	N		4/5	2/2			Burkholder (1965)	VII
Retina	A N	10/13		?	0/17		Coleman *et al* (1962)	XV
Pancreas	N	+		?			Coleman *et al* (1962)	XV
»	N	75/106	6/22	?	13/89	5/19	Berns *et al* (1964)	X
Placenta		26/29		?	1/34		Burstein *et al* (1963)	XI
Leg	A	14/26		?	9/59		Blumenthal *et al* (1966)	XII
Nervous system	N	+/6		?			Angervall *et al* (1965)	XVII

+ = Total number not stated
? = Not stated

COMMENTS TO TABLE 4

Reference Number

V, VII, X — See Comments to Table 2

XVI — Chronic glomerulonephritis (1) Chronic pyelonephritis (2)
Weakly positive Primary amyloidosis (1), Chronic pyelonephritis (2), rheumatoid arthritis (1)

XI — Rh incompatibility 1/9

normal and hyaline islets and in vessel walls in sections from both diabetic and non-diabetic subjects (Berns *et al*, 1964) In the diabetic kidneys the anti-insulin serum became localized into those sites where conjugated insulin was bound *in vitro* However, the percentage of positive results was less (Berns *et al* 1962) Burkholder (1965) made similar studies on specimens from necropsy kidneys from five diabetic subjects He found that the distribution of tissue bound anti insulin serum did not correspond very well with those regions where globulins were localized and where complement fixation *in vitro* was demonstrated

In most earlier studies listed in Tables 2 and 4, where the fluorescent antibody technique or the technique of studying the *in vitro* localization of conjugated insulin was used, the investigated specimens consisted of material obtained after necropsy or amputation, collected over long periods and probably under varying conditions Furthermore, in most of those specimens where specific fluorescence was obtained, marked pathological changes were demonstrated in the kidneys, diffuse or nodular glomerulosclerosis in the retinas, micro aneurysms in the islets of Langerhans hyaline changes and in amputated legs arteriosclerotic, ischemical or inflammatory changes Immunofluorescent observations made on these kinds of specimens must be judged with caution Some investigations dealing with this problem may be cited Moller (1961) was able to show that conjugated antisera were quickly localized intra cellularly in damaged cells Kent (1966, 1967) found that γG-globulin, albumin and fibrinogen were localized in ischemical heart muscle fibres both in experimental heart infarcts in dogs and in human infarcted heart tissue The proteins could not be eliminated by rinsing in buffered saline they behaved in an immunologically »bound« fashion Kent was also able to demonstrate the penetration of albumin into muscle fibres directly adjacent to blood vessels as a post-mortem phenomenon in undamaged tissue

Summary

Most studies cited in this chapter indicate that during the course of human diabetes mellitus morphological changes develop in small blood vessels throughout the body The changes are morphologically similar in different regions when studied under the light and electron microscopes, and their pathogenesis is probably similar However, it has not been possible to identify any structural or chemical patterns in the affected vessel walls by means of morphological, histochemical or chemical studies, which unequivocally separate the diabetic »microangiopathy« from changes observed in small blood vessels both in apparently healthy subjects and patients with some other diseases Nor has it been possible, with the aid of these studies and others not cited here, to elucidate the primary cause or causes of these vascular changes, on patients with »secondary« diabetes or on animals (*cf* Berkman & Rifkin, 1966)

To the present author it seemed of great interest that γ globulin (and components of complement) could be identified and found localized in these vascular changes in diabetic subjects The functions of these substances are fairly well known and their presence in vascular lesions in diabetes might be of etiological importance However, since most of these immunohistochemical studies have been performed on specimens obtained by necropsy or amputation, and since no extensive studies have been made of the distribution of γ globulin and other plasma proteins in the vessel walls of patients with short term diabetes and apparently healthy non-diabetic subjects, the importance of these observations is difficult to judge

CHAPTER II

EARLIER IMMUNOFLUORESCENT STUDIES ON THE DISTRIBUTION OF VARIOUS PLASMA PROTEINS IN THE HUMAN SKIN

As the major part of this investigation deals with the distribution of various plasma proteins in small dermal vessels in diabetic and non diabetic subjects, a short survey of earlier investigations, using the fluorescent antibody technique, of the distribution of plasma proteins in human skin will be given

Albumin was demonstrated by Gitlin *et al*, (1952) in the subepidermal connective tissue in normal skin Tissue obtained by necropsy was used A similar diffuse distribution in the superficial dermal layers was observed by Allansmith & Buell (1965) in skin biopsies from healthy and atopic individuals

Gitlin *et al*, (1952) also found fractions of β-globulins and fibrin localized extravascularly below the epidermis No other studies seem to have been published where the fluorescent antibody technique was used to study the distribution of different α- and β globulins in the normal skin, except for fractions of complement, *vide infra* Using immunoelectrophoresis, Zimmer *et al* (1960, 1961) found that the dermis contained α_1 and β globulins (three lines) together with γ globulin Fibrinogen was not observed in the normal skin by Burnham *et al*, (1963)

In apparently normal skin γG globulin has been found localized immediately below the epidermis unevenly and diffusely distributed in the connective tissue (Gitlin *et al*, 1952, Burnham *et al*, 1963, Larsson & Melin, 1964, Allansmith *et al*, 1964) Often a band of bright fluorescence indicating the presence of γ globulin was seen immediately beneath the epidermis, in the area of the dermal epidermal junction γA- and γM-globulins have been observed with a similar distribution by Allansmith & Buell (1965) These authors found no difference in the distribution in skin from normal and from atopic individuals

The presence of immunoglobulins and complement has been shown as characteristic narrow fluorescent lines in the area of the dermal epidermal junction in both apparently normal and pathologically changed skin taken from patients with systemic and discoid lupus erythematosus (Burnham et *al*, 1963, Cormane, 1964, Kalsbeek & Cormane, 1964, Cormane *et al*, 1966, Ten Have-Opbroek, 1966, Tan & Kunkel, 1966)

In the dermal vessel walls γ globulin was observed in skin obtained by necropsy (Gitlin *et al*, 1952), while Larsson & Melin (1964) did not find any fluorescence indicating the presence of this protein in two healthy individuals

Such a localization was observed, however, in patients with diabetes mellitus and systemic lupus erythematosus In the latter disease, it has also been found in skin with a typical rash by Tan & Kunkel (1966), and in skin from some patients with nodular vasculitis by Stringa et al, (1966), and Miescher et al, (1966)

Summary

See Chapter V

CHAPTER III

MATERIAL AND METHODS

Investigated subjects

Skin biopsies from 249 subjects were examined They include the following groups

A PATIENTS WITH DIABETES MELLITUS

Series A 50 patients These were selected from the files of the Medical Clinic, Umea, according to the following criteria about half had to be below the age of 40 and half above At the time of the investigation they had to be feeling well and their metabolic balance had to be acceptable They were called specially for this investigation to the Out patients Department

Series B 72 patients At the time of the investigation most of them were admitted to the Medical Clinic for regularization of their treatment or for other reasons In this group one biopsy specimen was lost and only incompletely studied (Biopsy No 1) In the final assessment of the result this patient was excluded

The object of choosing Series A was to try to get a sufficiently large group of diabetic subjects devoid of »secondary» factors (*e g* acidosis, infections, vascular complications such as myocardial infarction or peripheral gangrene, uraemia or oedema), which might influence the state of peripheral small vessels If only hospitalized patients were studied (Series B) the risk of such an influence seemed great

Information about patients with diabetes mellitus is given in Table 5

B MATCHED NON DIABETIC SUBJECTS

Each patient in Series A was matched with a non diabetic subject according to the following covariables age, sex and residence This was accomplished with the help of the official register of residents (»Folkbokforingsregistret») Individuals fulfilling the above criteria and registered close to the selected diabetic patients, were asked to cooperate in the investigation Three subjects refused one because of a disabling somatic disease, one because of nervous complaints, and one because of a fear of hospitals Another subject was excluded because of diabetes Instead, individuals registered next to these were chosen

All subjects in this series were investigated by the present author at the Out patients Department At the time of the investigation all felt well Slightly elevated blood pressure was recorded in six Three individuals complained of angina pectoris, one of chronic bronchitis, and three of recurrent urinary infections One woman was pregnant (beginning of third trimester), and one had been delivered of a healthy child without complications one week earlier

TABLE 5 *Patients with diabetes mellitus*

I The number age sex age range median age and known duration of diabetes me litus

Series	Sex	Number	Age range years	Median age	Known diabetes-duration 0—5	5—10	10—15	15- years
	Male	27	23—64	36	7	[illegible]	6	8
A	Female	23	18—75	34.5	5	8	6	4
	Total	50	18—75	35	12	14	12	12
	Male	34	16—80	47	18	3	[illegible]	5
[illegible]	Female	38	4—73	48	14	11	4	9
	Total	72	4—80	48	32	14	12	14
A+B	Total	122	4—80	43.5	44	28	24	26

II The number of patients who had been treated with insulin and who had vascular manifestations

Series			Known diabetes-duration 0—5	5—10	10—15	15- years
A+B	Insulin therapy	Number	15	23	21	23
		Per Cent	*34*	*82*	*87*	*88*
A+B	Vascular manifestations	Number	5	7	18	25
		Per Cent	*11*	*25*	*75*	*96*

C OTHER INVESTIGATED PATIENTS

77 patients with various diseases other than diabetes were investigated For different reasons (*cf* Introduction) patients with glomerulonephritis, systemic lupus erythematosus (SLE), rheumatoid arthritis and hypertensive vascular disease were chosen A few patients with other diseases and fairly healthy subjects with ill defined minor complaints were in most cases chosen at random for skin biopsy to form a basis of comparison with diabetic patients

Information about these patients is given in Table 6

Clinical and laboratory investigations

All subjects in this study were questioned about diabetic heredity and past or present diseases A general clinical examination was performed The blood pressure was recorded after at least five minutes rest and the figures thus obtained were used in the present study The eye grounds were examined by the present author If the results of an expert ophthalmological examination obtained less than one year ago, were available these were used Any microaneurysms with or without hemorrhages and exsudates in the eye grounds were noted as diabetic retinopathy Loss of reflexes or vibration sense in the legs was recorded The skin was examined for the presence of the atrophic circumscribed skin lesions described by Melin (1964) Nephropathy was registered in diabetic subjects if proteinuria was observed on repeated occasions and no reasons for kidney involvement other than diabetes were found Retinopathy

and nephropathy were summed up under the the common title »Vascular manifestations« in Table 5 The duration of any treatment with insulin was recorded It was thought very unreliable to divide diabetic patients into good or poor control groups

TABLE 6 *Other investigated patients*

Glomerulonephritis	9
Systemic lupus erythematosus (SLE) and SLE like syndromes	15
Rheumatoid arthritis	11
Hypertensive vascular disease	9
Other diseases	33
Total number	77

»Other diseases« Pyelonephritis (2) proteinuria (1) arteriosclerotic heart disease (2) pernicious anemia (1) chronic myeloid leukemia (1) amyloidosis (3) myasthenia gravis (2), hyperthyroidism (2) malabsorption (4) ulcerative colitis (2) cholecystitis (1) ill defined minor complaints (12)

In most investigated subjects, the haemoglobin values, the leukocyte counts and the values of blood sugar in peripheral blood (glucose oxidase method) were obtained The erythrocyte sedimentation rate according to Westergren — the ESR — for one hour was recorded The presence of proteinuria (Albustix®), glycosuria (Clinistix®) and in diabetic patients the total amount of urinary glucose throughout 24 hours, were investigated Microscopical examination of freshly voided urine was performed In the majority of subjects in series A and the matched non diabetic series the amount of serum protein was studied (paper electrophoresis)

By these investigations it was hoped to get a fairly good concept of the general health of the subjects studied

All the laboratory examinations were performed as ordinary routine samples by the Department of Clinical Chemistry of the University Hospital in Umeå

Histochemical and immunohistochemical investigations

A PERFORMANCE OF THE BIOPSIES AND HISTOLOGICAL PREPARATIONS

Punch biopsies with a diameter of 3 to 6 mm were taken from apparently normal skin from the lateral aspect of the proximal third of the lower leg

About half the biopsies were taken after local anesthesia with Xylocain® In the remaining subjects the area was sprayed with ethyl chloride to get anesthesia prior to the biopsy The small wound healed without complications even if the peripheral circulation was insufficient

Preliminary trials were made with quick frozen tissues, subsequently cut on a cryostat microtome However, it was not easy to obtain acceptable sections in this way Morphological details were not very distinct when immunofluorescent stainings were performed and the specimens could not be preserved for a long time Fixing in 10 % formalin or in 5 % glutaraldehyde was also tried In such sections the non specific fluorescence interfered when the fluorescent antibody technique was used In the main series a slight modification of a method introduced by Sainte Marie (1962) was chosen

All procedures, apart from the actual embedding in paraffin, were performed at about +4°C The small specimens were immediately washed in cold saline for a few seconds and carefully wiped dry of excess saline Large specimens were bisected Fixation was performed in cold 95 % ethanol for 24 hours After clearing in methyl benzoate (about 20 hours) and passage through xylene (3 hours) the specimens were embedded in paraffin The blocks were stored under refrigeration until used

The blocks were cut into 4—5 μ thick sections At least three sections were placed on every slide and stained in an identical fashion The cut sections were deparaffinized by passage for a few minutes, respectively, through two baths of xylene, then through 95 %, 85 % and 70 % ethanol Finally, the slides were washed for at least 20 minutes in two baths of buffered saline, pH 7 2 (0 01 M Na_2HPO_4 NaH_2PO_4 buffer + 8 5 g NaCl/100 ml)

All biopsies were stained with van Gieson s stain and with the PAS procedure (*cf* Lillie 1965)

B PERFORMANCE OF THE FLUORESCENT ANTIBODY TECHNIQUE

After removing excess fluid from the deparaffinized sections, the slides were placed in a moist chamber

Either »direct» or »indirect» immunofluorescent staining was used (*cf* Mellors, 1959, Nairn, 1962) The reactions took place at room temperature

The following table shows the principal procedures

After every application of antiserum, all unbound serum was carefully washed away in a large quantity of buffered saline (composition, see above) The sections were mounted in a »semipermanent» mounting medium (Rodriguez & Deinhardt, 1960) consisting of a solution of polyvinyl alcohol and glycerol in buffered saline, pH 7 2

The various *antigens* studied are listed in Table 7, and the unconjugated and conjugated *antisera* used in this investigation are listed in Table 8 Fluorescein isothiocyanate was used as a fluorechrome

The immunological specificity of the antisera was tested by the manufacturers In most antisera used it was confirmed by immunoelectrophoretic checks, kindly performed by Dr A Forsgren, Institute of Microbiology, University of Umea More sensitive tests were not carried out In practice it was thought improbable that weak cross reactions with undesired antigens would seriously influence the results of the immunofluorescent investigations

	Antigen to be demonstrated	Unconjugated antiserum 30 min	Saline pH 7 2 15 min	Conjugated antiserum 30 min	Saline pH 7 2 15 min	Mounting
Direct method	»A»	—	—	anti »A»	+	+
Indirect method	»A»	anti »A»	+	anti anti »A»	+	+

TABLE 7 Summary of the nomenclature and some quantitative data of the antigens (plasma proteins) studied by the aid of the fluorescent antibody technique

Nomenclature used	Synonymes	Approximate mean concentration in normal human plasma: Per cent of total protein	Mg per 100 ml normal plasma	Approximate Molecular weight	Approximate Sugar content: Total Per cent	Hexose Per cent
Albumin		55—64[1])	4500[4])	70 000[2])	0.5[3])	
α_1 acid glycoprotein	Orosomucoid	0.5[1])	75[4])	44 000[2])	41[3])	15[3])
α_1 lipoprotein	High density lipoprotein	3[1])	500[4])	165 000—400 000[6])		
Protein moiety				(23 000—28 000)×2—6[6])		
Haptoglobin		3—4[1])	100[4])	95 000[2])	22[3])	11.5[3])
Transferrin		3—6.5[1])	400[4])	90 000[2])	5.5[3])	2.5[3])
β lipoprotein	Low density lipoprotein	4—14[1])	600[4])	1.3—3.2×10[6] [6])		
Protein moiety				250 000—1000 000(?)[6])		
β_{1C} globulin	(A fraction of C'3 component of complement)		100[4])			
Fibrinogen			250[4])	340 000[4])	5[3])	3[3])
γA globulin[7])	IgA, β_{2A}, γ_1A		100[4])	160 000[4])		5[3])
γG globulin[7])	IgG, γ, 7Sγ, γ_2	10—17[1])	1000[4])	150 000[2])	3[3])	
γM globulin[7])	IgM, γ_{1M}, β_{2M}, 19Sγ		100[4])	1000 000[4])	10[3])	5[3])

EXPLANATIONS TO TABLE 7

1) cf Cooper, G. C., p. 88 in Putnam (1960)
2) cf Phelps, R. A. & Putnam, F. W., p. 158 in Putnam (1960)
3) cf Winzler, R. J., p. 309 in Putnam (1960)
4) cf p. 495 in »Något om blodäggvitan» AB Kabi, Stockholm 1966
5) cf Winzler, R. J., p. 235 in Siperstein et al (1964)
6) cf Fredrickson ■ al (1967)
7) cf Bull. World Health Organ. 30 447 1964

TABLE 8 *Antisera used in the fluorescent antibody studies*

A Unconjugated antisera

Serum No	Source (animal)	Specific human antigen	Cross reactions (immunoelectrophoresis)	Received from
1	Rabbit	Albumin	0	»SEVAC«[1]
2	»	»	0	Behringwerke[2]
3	»	α_1 acid glycoprotein	0	»
4	»	α_1 lipoprotein	0	»
5	»	Haptoglobin	0	»
6	»	Transferrin	0	»
7	»	β lipoprotein	0	»
[illegible]	»	β_{1C} protein	0	Dr H Muller Eberhard[3]
9	»	»	0	Dr B Lundh[4]
10	»	Fibrinogen	0	»SEVAC«
11	»	γG globulin	0	Umea[5]
12	»	»	0	Behringwerke
13	»	»	0	»SEVAC«
14	»	γA globulin	0	»
15	»	γM globulin	0	»

B Conjugated antisera

Serum No	Source (animal)	Specific antigen	Cross reactions (immunoelectrophoresis)	Received from
16	Rabbit	Human albumin		Behringwerke
17	Sheep	Human γ globulin	Albumin	SBL[6]
18	Rabbit	» »		SBL
19	Goat	» »	α- and β globulins (weak)	Microbiol Ass[7]
20	»	Rabbit globulin		» »

[1] »SEVAC« Prague, Czechoslovakia [2] Behringwerke Marburg Lahn, Western Germany [3] Scripps Clinic & Research Foundation La Jolla Calif USA [4] Department of Medicine University of Lund [5] Institute of Clinical Bacteriology University of Umea [6] Professor A. Fagraeus Statens Bakteriologiska Laboratorium Stockholm [7] Bethesda Md, USA

During the course of the study different antisera sometimes had to be used against the same antigen In such cases parallel stainings with both antisera were performed on a series of sections to get comparable results

The sections were examined in a Zeiss fluorescence microscope with dark field condenser and UG 2 exciter filter with a 41 barrier filter The light source was Osram s HBO 200 W mercury lamp For photomicrographs Ectachrome High Speed film (EH 135) was used with an exposure time of about 30 seconds

Unspecific fluorescence in the sections was checked in the following ways

In the *direct* procedure

1) The sections were covered for 20 minutes with an unconjugated antiserum. Most specific antigens in the sections were then blocked. Treatment with conjugated antiserum afterwards resulted in only weak or no fluorescence (Plate II B).

2) Specific antibodies in the conjugated antiserum were neutralized by coincidental application on the sections of both a solution of the specific antigen and the conjugated antiserum. After rinsing no specific fluorescence was present in the sections.

In the *indirect* procedure

Here only one conjugated antiserum was used in all tests, namely serum No 20 in Table 8.

3) The sections were only covered with the conjugated antiserum, *cf* Plate II D.

4) The sections were first covered with a normal rabbit serum for 30 minutes. After rinsing the conjugated serum was used.

Only in a few instances was a weak unspecific fluorescence noted in epidermis and in epithelial cells of dermal glands when methods 3 and 4 were used. No fluorescence was noted in vessel walls or in dermal connective tissue.

5) When the conjugated goat anti rabbit globulin serum was replaced by a conjugated rabbit anti goat globulin serum no fluorescence was obtained.

Most biopsies were stained by both direct and indirect methods to identify γG globulin. The histological pictures and the intensity of the specific fluorescence were in both instances very similar (Plate II). The same was found to be true when albumin was studied using both processes. The results were reproducible. Moreover, when unconjugated antisera against different antigens were used in the indirect methods, the results were dissimilar (Plates IV—V). This makes a high degree of immunological specificity probable.

However, when it is stated in the present investigation that γG globulin or other plasma proteins were demonstrated in the sections studied, it must be understood that the immunofluorescent »demonstration» or »staining» of these proteins (antigens) in reality means that conjugated antiserum could be observed specifically »bound» in the sections. The validity of the above-mentioned statements is dependent on the specificity of the antiserum and the possible presence of overlapping antigenic determinants in the tissues.

Semiquantitative grading of staining reactions in small dermal vessel walls

In this investigation the term »small blood vessels» includes capillaries, venules, and small veins localized immediately under the epidermis or close to dermal glands near the subcutaneous fat. The staining reactions in these vessels were similar. In several sections, both from diabetic and non-diabetic subjects, the immunofluorescent reactions in the small blood vessels differered from those observed in arterioles, small arteries, and larger arteries.

and veins (*cf* Plate VI C) Even in such sections, where strong specific fluorescence was noted in the walls of small vessels, other vessel walls were as a rule non fluorescent

The staining reactions in small dermal vessel walls were graded as follows

A. THE PAS PROCEDURE (Plate I)

Grade 1 In the majority of vessels the walls were thin with a delicate PAS positive reaction

Grade 2 In many small blood vessels a slight PAS positive thickening of the wall was observed

Grade 3 Most vessel walls were markedly thickened with a heavy PAS positive reaction

Grade 4 A heavy thickening of the majority of the vessel walls was seen, sometimes with a total obliteration of the lumen

Thus the semi quantitative grading of the wall thickness was similar to that adopted by Angervall and co-workers (*cf* Säve Söderbergh *et al* 1967) The reason why 4 grades instead of 5 — as used by these authors — were used in the main investigation was that the Sainte Marie fixation gave inferior histochemical results compared with *e g* the Bouin fixation permitting only a fairly reliable separation of 4 different stages In a pilot study (*cf* Sources of error) it was found that the outcome of the PAS procedure could be assessed in 5 grades but that this grading was less reproducible

B THE IMMUNOFLUORESCENT STAINING REACTION (Plate III)

Grade 1 No specific fluorescence was seen in vessel walls, or only traces in isolated vessels (— to ±)

Grade 2 A rather weak but distinct fluorescence was seen in disseminated small vessels A similar fluorescence could be observed in the surrounding connective tissue (+)

Grade 3 In many small blood vessel walls the fluorescence was distinct and bright Any fluorescence in the surrounding tissue was not so strong (++)

Grade 4 The fluorescence was very strong and bright in the majority of the observed small vessel walls It deviated distinctly from that observed in the surrounding tissue (+++)

STATISTICAL METHODS

The *methods of sampling* employed mean that the samples can be regarded as random samples from different populations The *populations* were

1 Patients with diabetes mellitus (Series A and B)
2 Matched non-diabetic subjects
3 Other investigated patients living in Umeå and its surroundings

The *findings* were as follows

1 The results of PAS staining and immunofluorescent demonstration of γG globulin and some other plasma proteins in small dermal vessel walls in a series of skin biopsies The reactions were graded semiquantitatively The results of both »open» and »blind» assessements were recorded

2 Some anamnestic clinical and laboratory data recorded in the series of subjects on whom the skin biopsies were made

The statistical *questions* to be considered were

Is there any difference between the immunofluorescent staining of sections prepared according to different methods (Chapter IV)? Are there any differences in the assessments of PAS treated sections by different investigators (Chapter IV)? Are there any differences in »open» and »blind» assessments of the immunofluorescent staining or in direct and indirect immunofluorescent stainings (Chapter IV)?

Most patients in the diabetes series B were hospitalized for various reasons while the subjects in the diabetes series A were not A comparison between the two series had to be made (Chapter IV)

Are there any differences in the staining reactions between the group of diabetic subjects and the group of matched non-diabetic subjects or between the latter group and the group made up of other investigated patients (Chapters VI—VIII)?

The possible influence of some anamnestic clinical and laboratory data on the staining reactions had to be examined (Chapters VI—VIII)

The *analytical methods* used were as follows

As a central value the mean with its standard deviation($s_{\bar{x}}$) was employed This and the confidence interval were calculated according to current formulas (cf Documenta Geigy 1960) The difference between two population means was tested by means of a *sign test* or a Student s t test The critical values depending on the level of significance and the number of degrees of freedom were taken from current tables When the computed value coincided with or was greater than that given in the table the difference was statistically significant The possible influence of some data on the staining results was tested by the Chi square method (χ^2) A few hypotheses were tested by means of a one way analysis of variance To test the hypothesis that different investigators differ in their assessments of the staining reactions the effect of differences between patients had to be reduced Thus the comparison was carried out with a two-way analysis of variance

The test methods used are given in the various chapters The *critical value* is given in brackets after each computed value, or in the headings of the tables

All tests were performed at the 5 % *significance level*

Summary

A clinical examination was performed on 122 patients with diabetes mellitus Of these 50 were specially called to the Out patients Department A contrasting sample of 50 non diabetic apparently healthy subjects was selected with the help of the official register of residents, and studied in the same way as the diabetic patients Furthermore, 77 hospitalized patients with various diseases other than diabetes mellitus were studied Some laboratory examinations were performed in order to obtain a fairly accurate concept of the general health of the individuals studied

From all these 249 subjects punch biopsies were taken from apparently normal skin on the lower leg Sections from these biopsies were prepared and stained by van Gieson s stain and by the PAS procedure Other sections were studied by means of the fluorescent antibody technique The performance of this method is described The primary objective was to identify, with this technique, the possible localization of various plasma proteins in the walls of small dermal vessels

The results of the PAS treatment and the immunofluorescent stainings were recorded according to a semiquantitative grading The statistical methods used in the further estimation of the results are described

CHAPTER IV

SOURCES OF ERROR

Possible sources of error in the technical performance

The same region of the lower leg was chosen throughout the whole investigation for the skin biopsies in order to exclude regional variations

Some idea of the magnitude of this source of error was given by a pilot study of eight patients with diabetes One skin biopsy was taken from the lower leg and another from the shoulder region Sections from both the biopsies were treated in an identical fashion and simultaneously stained by the PAS procedure and with the indirect method of identifying γG globulin (*cf* Table 9) In four patients the PAS stained vessel walls were judged as one grade thinner in the skin from the shoulder region than in that from the leg A similar one grade difference was noticed in two patients in immunofluorescence stained sections These differences are rather subtle and barely within the limits of the subjective estimation methods used

Similarly, in a few patients a comparison was made between biopsies obtained after local anesthesia with Xylocain[a] and with ethyl chloride No differences in the staining reactions were noted

In 12 patients a comparison was made between skin biopsies which were quick frozen and cut on a cryostat microtome washed in buffered saline pH 7 2, and finally treated for 10 minutes with 95 % ethanol prior to the immunofluorescence staining and skin biopsies treated as described before according to the modified Sainte Marie method, Table 10 When the first method was used, the fluorescence indicating the presence of γG globulin in the vessel walls was weaker (1—2 grades) in eight patients The difference was statistically significant (the sign test)

TABLE 9 *Comparison between the staining reactions (PAS staining and immunofluorescent γG globulin staining) in small vessels in skin biopsies taken from the shoulder region and the lower leg in patients with diabetes mellitus*

Patient No	PAS staining Shoulder	PAS staining Leg	γG globulin staining Shoulder	γG globulin staining Leg
4	4	4	4	4
23	3	3	4	4
169	1	2	2	3
170	1	2	2	3
171	1	2	1	1
173	2	2	3	3
184	3	3	4	4
185	2	3	2	2

This may be explained partially by the greater difficulty in cutting thin

sections from frozen tissues, and the histological details in these sections were indistinct (cf Sainte Marie, 1962, Farrant & Shedden, 1965) Furthermore, in quick frozen unfixed sections most soluble proteins may be washed away prior to the immunofluorescence staining The staining then in the main demonstrated »bound» proteins

In this study cold ethanol was used as a fixing fluid In most histological work this is not an ideal substance to choose It slowly penetrates the tissues giving them a tendency to shrink and harden and this may cause artifacts However, the histological similarities of the immunofluorescence stainings in alcohol fixed and in quick frozen blocks seem to indicate that such artifacts did not seriously affect the results Alcohol is also said to be a useful fixative for preserving many proteins (Lillie, 1965), and it has been used earlier in immunohistochemical studies similar to those reported here (Mellors, 1959, Nairn, 1962, Brandzaeg *et al* 1965, 1967)

TABLE 10 *Direct immunofluorescence staining with goat anti human globulin serum The results of a comparative study between quick frozen washed skin sections and sections treated according to the modified Sainte Marie method*

No	Immunofluorescence in small vessels (Grade 1—4) Frozen sections (cryostat microtome technique)	Paraffin embedded sections (Sainte-Marie method)
1	2	4
2	1	3
3	2	3
4	2	3
5	3	3
6	1	1
7	1	3
8	1	1
9	1	3
10	1	2
11	2	2
12	2	3

Errors in the semiquantitative grading

The biopsy specimens were numbered in chronological order irrespective of diagnosis Generally two to four weeks elapsed between the time of biopsy and the study of the slides under the fluorescence microscope, and another four to eight weeks before the PAS studies were made By timing the microscopic examinations thus an attempt was made to prevent knowledge of the diagnosis and the results of the earlier stainings influencing the interpretation of the results Nevertheless, the microscopic studies must be considered »open» studies, prejudiced by the investigator s knowledge of the source of the material and his desire to achieve some definite result from the study It therefore seemed important to try to observe if there were any differences between »open» and »blind» assessments of the staining reactions

A. THE ASSESSMENT OF THE WALL THICKNESS

»Blind» assessments of 23 slides with PAS treated sections were made, independently of each other, by three

pathologists[1] The changes observed in small blood vessels were graded from 1—5 in a scale previously adopted in similar studies by Angervall and coworkers (cf Säve-Söderbergh *et al*, 1967) The present author made an independent »open» assessment of the same sections using the same scale The results are given in Table 11

A two-way analysis of variance was made for testing if there were any differences in the expected mean values of the four independent investigators A significant difference was obtained $F = 4.6$ ($F_{(3,66)} = 2.8$)

As can be seen from the Table however, there was never a difference of more than one grade between the assessments of the individual sections by the four observers The results seem to indicate that the present author's assessment of the staining reactions was not seriously prejudiced by his knowledge of the source of the material studied As pointed out earlier, a one grade difference in the assessments is a subtle one and barely within the limits of discrimination of this subjective estimation method The processing of the specimens according to Sainte-Marie (1962) did not give ideal conditions for performing the PAS procedure This fact may also be responsible for the individual variations noted As pointed out in Chapter III a four grade semiquantitative scale was chosen in the final assessment of the wall thickness in this study

[1]) Drs. L. Angervall and J Säve-Söderbergh Institute of Pathology Göteborg and Dr S Falkmer Institute of Pathology II Umeå

TABLE 11 *The grading of 23 PAS-treated sections from skin biopsies by four independent investigators A—D The source of the material was known to A (»open» study) but unknown to the others (»blind» study)*

Slide No	PAS-staining Grades 1—5 »Open» A	»Blind» study B	C	D
1	4	3	4	4
2	1	1	2	1
3	1	2	2	
4	2	2	2	2
5	1	2	2	1
6	4	5	5	[illegible]
7	2	3	3	3
8	5	5	5	[illegible]
9	3	2	3	2
10	1	1	1	1
11	1	2	1	1
12	1	2	1	1
13	3	3	3	3
14	3	3	3	3
15	3	3	3	3
16	3	4	3	3
17	2	2	3	2
18	2	2	3	2
19	3	2	3	2
20	2	2	2	2
21	4	3	4	4
22	3	3	3	2
23	3	4	4	3
Total	57	60	65	56

Lastly the results of this »open» and »blind» study confirm the previous observations by Angervall *et al* (1965) that pathologists (and clinicians) with experience of studying histological changes in skin sections only differ slightly in their estimation of the thickness of small vessel walls in PAS-treated light microscopic slides when a semiquantitative grading is used

B THE ASSESSMENT OF THE IMMUNOFLUORESCENT STAINING

Newly cut sections were prepared from all skin biopsies in the diabetes mellitus series A and from the series of matched non diabetic subjects The slides were codified and stained in the following manner

A Direct immunofluorescent staining Conjugated goat antihuman globulin serum (serum No 19, Table 8) was used

II Indirect immunofluorescent staining First layer Rabbit-antihuman γG globulin serum (serum No 13, Table 8)
Second layer Conjugated goat anti-rabbit globulin serum (serum No 20, Table 8)

The specific fluorescence in small dermal blood vessel walls was graded as described above (Grades 1—4)

A »blind» study of the slides was made, and the results then compared

TABLE 12 *Matched non diabetic subjects The results of the »open» and »blind» interpretations of the immunofluorescent demonstration of γG globulin in small dermal vessel walls*

Interpretation	Immunofluorescence method	Group No	Fluorescence Grade 1	2	3	4
Open	Direct	I	32	14	4	0
Open	Indirect	II	35	15	0	0
Blind	Direct	III	32	8	7	3
Blind	Indirect	IV	32	14	3	1

Groups compared	Chi square value	($t_{0 025}$ = ± 2 0)
I and III	2 4 (7 8)	<1 0
II and IV	3 9 (6 0)	1 7
I and II	1 2 (3 8)	1 7
III and IV	4 2 (7 8)	1 0

TABLE 13 *Diabetes mellitus series A The results of the »open» and »blind» interpretation of the immunofluorescent demonstration of γG globulin in small dermal blood vessels*

Interpretation	Immunofluorescence method	Group No	Fluorescence (Grade) 1	2	3	4
Open	Direct	I	11	17	17	5
Open	Indirect	II	13	16	11	10
Blind	Direct	III	12	15	19	4
Blind	Indirect	IV	15	15	11	9

Groups compared	Chi square value ($\chi^2_{0 05}$ = 7 8)	t value ($t_{0 025}$ = ± 2 0)
I and III	0 4	<1 0
II and IV	0 2	1 0
I and II	3 1	0
III and IV	4 4	0 4

TABLE 14 *Diabetes mellitus series A and the series of matched non-diabetic subjects. Results of the »open» and »blind» interpretation of the direct and indirect immunofluorescent staining to demonstrate localized γG-globulin in small dermal vessel walls in individual subjects. The results of the PAS-staining are also given*

	Diabetes mellitus series A						The series of matched non-diabetic subjects					
		Immunofluorescence Grade 1—4						Immunofluorescence Grade 1—4				
		»Open»		»Blind»		PAS		»Open»		»Blind»		PAS
Pair No	Pat No	Dir	Indir	Dir	Indir	Grade 1—4	Pat No	Dir	Indir	Dir	Indir	Grade 1—4
1	4	4	4	3	4	4	133	1	1	1		
2	5	2	2	4	3	3	22[illegible]	1	1			
3	17	3	3	2	3	3	223	1	1			1
4	25	4	3	3	4	4	14	1	1	1		
5	34	3	4	4	2	2	225	1	1			
6	56	3	4	1	1	3	1[illegible]	1	1	1		
7	64	4	4	3	4	2	2[illegible]0	2	2			
8	66	4	3	2	3	3	1[illegible]0	1	1	1		1
9	67	1	2	1	2	3	1[illegible]2	1	1	1		
10	80	3	3	2	1	3	15	2	1	1	1	2
11	89	3	4	3	4	3	1[illegible]3	3	2	1		
12	113	2	2	2	2	3	19	1	1	1	2	1
13	126	2	3	4	4	4	1[illegible]1	1	1		1	
14	123	4	4	2	1	1	137	1	1	1	2	1
15	130	3	4	2	4	3	129	2	2	2	2	1
16	131	2	3	2	2	3	134	1	2	1		2
17	137	2	3	3	4	4	140	2	2	1	2	
18	138	3	3	3	4	4	1[illegible]4	3	1	1	1	1
19	142	3	4	3	3	3	145	1	1	1	1	1
20	143	2	1	3	3	1	144	1	1	1	2	1
21	145	1	1	1	3	1	190	1	2	1	1	2
22	147	1	3	3	2	3	141	1	2		2	1
23	161	1	1	2	3	2	168	2	1	3	3	1
24	162	2	1	1	1	2	163	1	1	1	1	2
25	164	2	2	2	2	2	169	2	1	4	1	1
26	165	2	1	1	1	2	157	2	2	3	1	2
27	172	3	4	4	2	1	174	1	1	2	1	2
28	175	3	2	2	2	2	160	2	2	2	3	1
29	176	3	3	3	1	1	179	1	1	1	1	2
30	177	2	2	3	1	2	1[illegible]9	1	2	1	1	2
31	181	2	2	3	4	2	193	1	1	1	2	2
32	182	1	1	2	2	2	193	1	1	2	2	1
33	184	3	4	2	3	3	199	1	1	2	1	1
34	185	2	2	2	2	3	1[illegible]9	2	2	4	4	2
35	187	1	1	1	1	1	214	1	1	4	1	2
36	189	2	2	3	3	3	197	1	1	4	1	1
37	194	2	2	3	1	2	156	2	1	2	1	2
38	196	1	2	1	2	3	193	1	1	3	3	1
39	197	3	2	3	2	2	216	3	2	2	1	3
40	204	3	1	3	1	2	191	2	1	3	1	2
41	209	1	1	1	3	2	207	1	1	1	1	2
42	210	3	1	3	1	1	224	3	1	3	2	3
43	211	1	[illegible]	1	2	2	219	1	1	1	1	2
44	212	2	2	1	2	2	220	1	1	1	1	1
45	213	2	1	3	1	2	215	2	2	1	1	1
46	221	1	1	[illegible]	3	1	228	1	1	1	2	2
47	227	3	2	1	1	1	233	1	1	1	1	1
48	230	3	3	2	2	2	237	1	1	1	[illegible]	[illegible]
49	236	2	2	3	1	3	23	2	2	3	1	3
50	239	1	1	1	1	2	242	[illegible]	2	3	2	2

Dir = Direct staining Indir = Indirect staining

with the results of the earlier »open» study of identically stained slides from the same biopsy specimens As can be seen from Tables 12 and 13, no significant differences were found between the »open» and »blind» interpretations of the staining reactions in the two series Therefore, it seems probable that the »open» interpretation of the immunofluorescent stainings in the two series was not seriously prejudiced by the author s knowledge of the source of the material under investigation

The results for the individual subjects in the study are listed in Table 14

A comparison was also made between the conclusions obtained by »open» and »blind» interpretations of the direct and indirect stainings in the two series No significant differences were found Although the antiserum used in the direct staining gave weak cross reactions with globulins in the α- and β-groups when tested with immunoelectrophoresis (Table 8), it seems probable that the fluorescence mainly indicated localized γG-globulin in tissue sections

As stated previously, the patients with diabetes mellitus were divided into

TABLE 15 *Patients with diabetes mellitus Comparison between the results of the PAS stainings and the indirect staining of γG globulin in series A and B The immunofluorescence staining is also compared in the two series in sub groups with different duration of the disease*

Series of subjects studied	Group No	PAS-staining (Grade) 1	2	3	4
Series A	I	9	19	17	5
B	II	19	30	17	5
		γG globulin staining (Grade)			
A	III	13	16	11	10
B	IV	26	19	21	5
A (Duration 0— 5 years)	V	7	5	11	0
B » » »	VI	17	4	8	1
A (Duration 5—10 years)	VII	4	5	2	3
B » » »	VIII	4	[illegible]	0	2
A (Duration 10—15 years)	IX	2	2	4	4
B » » »	X	3	3	6	0
A (Duration 15— years)	XI	0	4	5	3
B » » »	XII	2	3	7	2

Series compared	Chi-square value
I and II	2 8 (7 8)
III and IV	5 9 (7 8)
V and VI	0 1 (3 8)
VII and VIII	0 1 (3 8)
IX and X	0 7 (3 8)
XI and XII	0 1 (3 8)

two series, A and B, *cf* Chapter III A comparison was made between the PAS stainings in the two series, and between the »open« interpretation of the indirect γG globulin stainings in series A and B No significant differences were obtained The two series were also divided into four sub groups according to the duration of the disease, and the γG globulin stainings of the two series in each sub group were compared No significant differences were obtained

The results are listed in Table 15

Summary

Some possible errors in the technical performance of the staining procedures and the assessment of the results of the stainings were investigated

Significant differences were found between the results of the immuno fluorescent staining of sections prepared according to the cryostat microtome technique and according to the Sainte-Marie method Specific fluorescence was seen more often when the latter method was used No significant difference was found when the staining reactions in sections from [illegible] taken from the lower leg were compared with those from the shoulder region

When PAS treated sections were assessed by four different investigators independent of each other some differences in the results were obtained The assessment of individual sections however, differed only at the most one grade, when a five graded scale was used

An »open» assessment of immuno fluorescence stained sections was compared with a »blind» one There were no significant differences between the results of the two assessments, neither in the series of diabetic patients, nor in the series of non diabetic subjects

CHAPTER V

THE RESULTS OF THE IMMUNOFLUORESCENT LOCALIZATION OF VARIOUS PLASMA PROTEINS IN THE SKIN, APART FROM THE SMALL VESSELS

In the present investigation the distribution of various plasma proteins in different anatomical sites in the skin was studied both in healthy subjects and in patients with diabetes Some patients with other diseases were also studied The observations in small vessel walls will be dealt with in more detail in Chapters VII and VIII

Healthy Subjects

The results are summarized in Table 16

1 ALBUMIN

49 subjects A sometimes bright fluorescence was unevenly distributed within the superficial layers of the dermis It seemed to be localized around connective tissue fibres Sometimes a more flocculent distribution was noted Areas with bright fluorescence could be seen peripherally around groups of small vessels Not infrequently, a condensation of fluorescence — sometimes as a band — was observed immediately below the epidermis

2 α_1 ACID GLYCOPPOTEIN

In most of the 21 subjects studied, no fluorescence was observed In six subjects a rather weak fluorescence was noted in the cytoplasm of small groups of cells within, or close to, the walls of small superficial vessels

3 α_1 LIPOPROTEIN

31 subjects The distribution of specific fluorescence paralleled that observed for albumin, but it was in general weak Sometimes no fluorescence was observed, or only traces of it In larger vessels a very delicate, fluorescent network was often seen around the endothelial cells Traces of fluorescence could be seen sometimes in the deeper layers of the walls in these vessels

4 HAPTOGLOBIN

20 subjects No fluorescence was observed except occasionally as traces immediately below the epidermis or coating the endothelial cells in blood vessels

5 TRANSFERRIN

13 subjects The extravascular distribution was very similar to that of albumin, but the fluorescence was weak

6 β LIPOPROTEIN

19 subjects No fluorescence was noted outside the blood vessels A thin fluorescent rim could be seen coating the endothelial lining in blood vessels

TABLE 16 *Healthy subjects Distribution of various plasma proteins in different dermal structures Fluorescent antibody technique*

		Albumin	α1 acid glycoprotein	α1 lipoprotein	Haptoglobin	Transferrin	β lipoprotein	β1c globulin	Fibrinogen	γG globulin	γA globulin	γM globulin
Epidermis		− / +−	−	−	−	−	−	−	−	− / +−	−	−
Dermal epidermal junction		− / +	−	− / +−	−	− / +−	−	−	−	+ / ++	− / ++	− / +−
Connective tissue	Superficial	+ / +++	−	− / +−	−	− / +	−	−	−	+− / ++	− / +−	− / +−
	Deep layer	− / +	−	− / +−	−	− / +−	−	−	−	− / +−	−	−
Glands & Muscles		−	−	−	−	−	−	−	−	−	−	−
Glandular basement membr		−	−	−	−	−	−	−	−	− / +−	−	−
Plasma cells		−	−	−	−	−	−	−	−	− / +	−	−
Small blood vessels	Endothelium	− / ++	− / +−	− / +	− / +	− / +	− / +−	− / +	− / ++	− / +	− / +−	− / +−
	Vessel wall	− / +	−	− / ++	− / +−	− / +−	− / +−	− / +	− / +−	− / ++	− / +	− / +
	Perivascular cells	−	− / +−	−	−	−	−	−	−	−	−	−
Larger vessels Arteries	Intima	− / +−	−	+ / ++	− / +−	− / +−	− / +−	− / +−	− / ++	− / +−	−	−
	Elastic membrane	−	−	−	−	−	−	−	−	−	−	−
	Media	−	−	− / +−	−	−	−	−	−	−	−	−
Larger vessels Veins	Intima	− / +	−	− / +	− / +−	− / +−	− / +−	− / +−	− / +	−		−
	Media	− / +	−	− / +	−	− / +−	−	−	−	−	−	−

− = No fluorescence
+− = Traces of fluorescence
+ = Weak fluorescence
++ = Distinct fluorescence
+++ = Bright fluorescence
− / +++ = Range of fluorescence in various biopsies

7 β_1c GLOBULIN

44 subjects In some specimens traces of fluorescence were observed lining endothelial cells and in the area of the dermal-epidermal junction

8 FIBRINOGEN

46 subjects No fluorescence was seen outside blood vessels A network of delicate fluorescence could be found around endothelial cells in larger vessels

9 γG GLOBULIN

50 subjects In a few specimens almost no fluorescence was observed in the dermis In most investigated biopsies an unevenly distributed fluorescence was noticed in the dermal connective tissue The distribution was similar to that observed when albumin was studied In general, however, the fluorescence was weak Often, it was only observed immediately below the epidermis as a condensation band quickly fading into deeper layers Small areas with specific fluorescence might be surrounded by zones without any fluorescence A weak fluorescence could be observed in the basement membranes of dermal glands An exceptional finding was some cells with fluorescent cytoplasm probably representing plasma cells

10 γA GLOBULIN

In most of the 39 subjects studied no fluorescence was present in the superficial layers of the dermis, or only traces of it Quite often the dermal-epidermal junction fluoresced within restricted areas as a very narrow, well defined band

11 γM GLOBULIN

44 subjects Only in exceptional cases was a rather dull fluorescence noticed in the superficial layers of the dermis

Patients with diabetes mellitus

With a few exceptions no essential differences were observed in the distribution and intensity of the fluorescence indicating the presence of the various plasma proteins in healthy subjects and in apparently normal skin from patients with diabetes

It was the author s impression that *the fluorescence indicating γG globulin was more often observed in the* dermal-epidermal junction in diabetic than in healthy subjects Similarly, it was often typical of diabetes mellitus that the basement membrane around sweat glands fluoresced distinctly This basement membrane also seemed abnormally thick *More often than in healthy* subjects clusters of fluorescent plasma cells was noted around small vessels

Other patients

Especially intense fluorescence indicating the presence of albumin could be seen in a few skin sections from oedematous patients

Plasma cells were rather frequently noticed, sometimes abundantly, in patients with rheumatoid diseases and systemic lupus erythemathosus Only in two patients with the latter disease were observations made of intranuclear fluorescence indicating γG globulin in the epidermis (This was also the case with one patient with long term dia

betes) A narrow line of heavy fluorescence was often seen in the dermal epidermal junction in skin taken from patients with systemic lupus erythematosus and a few patients with rheumatoid arthritis The boundaries between this observation and the findings in healthy subjects were indistinct It was the author s impression that the fluorescence indicating the various immunoglobulins was brighter and more often generally distributed in the dermal connective tissue from patients with rheumatoid diseases

The distribution of some of the plasmaproteins in the skin is given in Plates IV—VI

Summary

There were no distinct histological differences in the distribution of the various plasma proteins studied in skin from healthy subjects, from patients with diabetes and from patients with some other diseases The often disseminated distribution of specific fluorescence in the connective tissue in individual s [illegible] made any quantitative assessment [illegible] the fluorescence difficult Fluorescen[illegible] indicating the presence of some pro[illegible] as a constant finding in the s[illegible] layers of the dermis, whilst f[illegible]nce indicating the presence of [illegible] ers was an exceptional observat[illegible] Table 16

The ob[illegible]tions reported here on the distr[illegible] of albumin, β_{1C} globulin, f[illegible] and the various γ globulins [illegible] human skin are in the main iden[illegible] with those reported by other au[illegible] earlier (Chapter II) Minor differences might be due to differences in the technical performance of the immunofluorescent staining (*cf* Chapter IV)

CHAPTER VI

THE RESULT OF THE PAS STAINING OF SMALL DERMAL VESSEL WALLS

Morphology

The histological appearance of the small blood vessels in PAS treated sections did not deviate from the picture which other authors have given previously A survey of these findings is given in Chapter II Suffice it to say that the study concentrated on the variations in thickness of the vessel walls Any possible changes in the endothelial cells were not impressive and the pericytes were not studied in any detail Plate I illustrates the various grades of PAS staining

Matched non diabetic subjects

In the series of matched non diabetic subjects it was not possible to show any significant differences in the PAS staining between individuals below the age of 40 and those who were 40 years old or more (Nor was any significant difference found, when subjects below the age of 30 were compared with those above the age of 50) When males and females were compared no significant differences were observed (Table 17)

The possible influence of the blood pressure on the PAS staining was studied Table 18 gives the mean systolic and diastolic blood pressure in the various groups of PAS staining The expected means were compared by the aid of a one way analysis of variance No significant differences between the expected mean pressures were observed [Systolic pressure $F = 0\,7$, diastolic pressure $F = 0\,6$ $F_{(2\,47)} = 3\,2$)]

TABLE 17 *Matched non diabetic subjects The results of the PAS staining in subjects < 40 years and ≥ 40 years of age and in males and females*

Series of subjects studied	PAS staining (Grade) 1	2	3	4	Total No/ series	Chi square value ($\chi^2 = 6\,0$)	t value ($t_{0\,025} = \pm 2$)
< 40 years	18	10	1	0	29	2 0	1 5
≥ 40 years	9	10	2	0	21		
Males	13	12	2	0	27	0 7	1 2
Females	14	8	1	0	23		
Total No/Grade	27	20	3	0	50		

TABLE 18 *Matched non diabetic subjects Systolic and diastolic blood pressure in different groups of PAS staining*

PAS staining (Grade)	Total No/Grade	Mean systolic pressure $\pm s_{\bar{x}}$	Mean diastolic pressure $\pm s_{\bar{x}}$
1	27	141 ± 6	87 ± 2
2	20	139 ± 6	86 ± 2
3	3	155 ± 19	93 ± 2
4	0	—	—
Total No	50		

Patients with diabetes mellitus

The possible influence of some factors of the diabetic disease on the wall thickness of small dermal vessels was studied The results are listed in Table 19 They may be summarized as follows

Thickened vessel walls were more frequently found in diabetic patients than in apparently healthy non diabetic subjects (Test 1 and 2)

In the non diabetic series eight subjects knew of diabetes among close relatives This group however, did not significantly deviate from the group of subjects without any known occurrence of diabetes among relatives (Test 3)

The diabetic patients were divided into four sub groups according to the known duration of the disease Significant differences in wall thickness between the various groups were observed (Test 4) The wall thickness increased with the mean duration of the disease (Figure 1)

Patients with a known duration of less than five years did not deviate significantly in wall thickness from non diabetic subjects (Test 5) whilst in patients with a duration of less than 10 years the differe (Test 6)

When diabetics tion of less than 1 into two groups ac onset of the diseas after the age of difference betwee two groups was patients with a l disease below the grade 3 or 4 thic positive vessel wa first after eight ye betics with a kno of 40 or more a s noted already wit 2) Thus the age disease was of sor results In the gro known onset at th a comparison wa tients with a kno five years and tho five years or more ence was obtained The influence of thickening of the difficult to show onset diabetes

TABLE 19 *The results of the PAS staining of small dermal blood vessels in patients with diabetes mellitus The results were compared with those for the matched non diabetic series The influence of known familial occurrence of diabetes was studied in the non diabetic series In the diabetic series the influence of the known duration of the disease the age at the onset of the disease and the influence of insulin treatment were studied*

Series of subjects studied	Group No	PAS staining (Grade) 1	2	3	4	Total No
Matched non diabetic subjects	I	27	30	3	0	50
Known familial occurrence of diabetes	II	4	3	1	0	8
Familial occurrence not known	III	23	17	2	0	42
Diabetes mellitus Series A	IV	9	19	17	5	50
Diabetes mellitus Series B	V	19	30	17	5	71
Diabetes mellitus Series A + B	VI	28	49	34	10	121
Duration 0—5 years	VII	14	21	6	1	42
Duration 5—10 years	VIII	8	14	6	1	29
Duration 10—15 years	IX	4	7	11	2	24
Duration 15— years	X	2	7	11	6	26
Onset < 40 years duration < 10 years	XI	10	15	4	1	30
Onset ≥ 40 years duration < 10 years	XII	12	20	8	1	41
Insulin treatment diabetes < 5 years	XIII	5	7	3	0	15
No insulin treatment diabetes < 5 years	XIV	9	14	3	1	27

Test No	Groups compared	Chi square value	t value	Sign test value
1	I and IV	238 (78)		30 (25)
2	I and VI	226 (78)	70 (20)	
3	II and III		07 (20)	
4	VII VIII IX and X	224 (126)		
5	I and VII	51 (60)		
6	I and VII+VIII	83 (60)		
7	XI and XII	03 (60)		
8	XIII and XIV	0 (38)		

FIGURE 1 *Patients with diabetes mellitus The results of the PAS staining of small dermal blood vessels are plotted against the known duration of the disease (logarithm scale) The mean duration and the confidence limits of the mean in each grade of PAS staining are shown The values are*

PAS staining (Grade)	No	Mean duration (Years)	95 % confidence limits (Years)
1	28	58 ± 10	38 — 78
2	49	78 ± 11	56 — 100
3	34	130 ± 14	102 — 158
4	10	180 ± 31	111 — 249

The possible influence of insulin treatment on the development of the PAS positive changes in small vessel walls was studied in diabetics with a known duration of less than five years There was no significant difference when this group was compared with a similar group of diabetics who had not received insulin treatment (Test 8)

Patients with other diseases than diabetes mellitus

The wall thickness of small dermal vessels was studied in 77 hospitalized patients with diseases other than diabetes The results are listed in Table 20 In some groups the number of patients investigated was small The results must therefore be judged with great caution

Only in the series of patients with systemic lupus erythematosus or SLE like syndromes and in patients with rheumatoid arthritis was a significant difference obtained when the groups were compared with the series of matched non diabetic subjects In the statistical treatment of the results, grades 2—4 were combined because of the smallness of the series

Summary

In a series of apparently healthy subjects a slight thickening of the PAS positive vessel walls was seen in 40 % and markedly thickened walls were noted in 6 % of the investigated skin biopsies In a contrasting series of diabetic patients a slight thickening was seen in 38 % and a marked or heavy thickening in 44 % of the studied biopsies The difference was statistically significant

There was no positive influence of age, sex or blood pressure on the wall thickness of the vessels

FIGURE 2 *Patients with diabetes mellitus divided into three groups according to the known age at the onset of the disease The results of the PAS staining of small dermal vessels are plotted against the known duration of the disease (logarithm scale) The mean duration and the confidence limits of the mean in each grade of PAS staining are shown The values are*

Known onset below the age of 20

PAS staining (Grade)	No	Mean duration (Years)	95 % confidence limits (Years)
1	9	8 ± 20	34 — 126
2	13	132 ± 26	74 — 186
3 + 4	20	18 ± 18	142 — 218

Known onset at the age of 40 or more

PAS-staining (Grade)	No	Mean duration (Years)	95 % confidence limits (Years)
1	14	42 ± 1	20 — 64
2	24	43 ± 09	24 — 62
3 + 4	15	9 ± 19	5 — 13

TABLE 20 *The results of the PAS staining of small dermal blood vessels in patients with diseases other than diabetes mellitus The results were compared with those of the matched non diabetic series The various diagnoses in the group »Other diseases» are listed in Table 6*

Group of patients studied	PAS staining (Grade) 1	2	3	4	Total No/Group	Chi square value (x = 3 8)
Glomerulonephritis	4	3	2	0	9	0 3
SLE syndrome	3	10	2	0	15	5 4
Rheumatoid disease	2	5	4	0	11	4 6
Hypertension (Mean 221/123)	6	2	0	1	9	0 5
Other diseases	21	11	1	0	33	0 8
Matched non-diabetics	27	20	3	0	50	

In the diabetic series it was noted that the width of the PAS-positive vessel walls increased with the duration of the disease This was a statistically significant finding in juvenile diabetics with a known onset below the age of 20 In patients with a known onset at age of 40 or more there was no statistically significant difference between patients with a known duration less than five years and those with a duration of five years or more

It was not possible to show any influence of known diabetic heredity or of insulin treatment on the vascular changes

In two groups of patients with SLE and rheumatoid arthritis the vessel walls were significantly thicker than in apparently healthy subjects

CHAPTER VII

THE RESULTS OF THE IMMUNOFLUORESCENT LOCALIZATION OF γG GLOBULIN IN SMALL DERMAL BLOOD VESSELS

Morphology

The specific fluorenscence indicating localized γG globulin in the small dermal blood vessels was seen in the region of the basement membrane outside the non fluorescent endothelial cells Sometimes it was seen as a single delicate band, sometimes this was split up into two or more fluorescent layers In some cases the whole periendothelial wall fluoresced homogenously and sometimes only part of the vascular circum ference fluoresced As a rule both groups of non fluorescent vessels and vessels with various grades of fluorescence were seen in the same sections The intensity of the specific fluorescence varied significantly in various groups of investigated subjects Unless otherwise stated only the results of the indirect immunofluorescent staining are discussed The morphological picture of direct and indirect fluorescent antibody staining were practically identical

Matched non-diabetic subjects

The γG globulin staining was analyzed in the same way as the results of the PAS treatment (*cf* Chapter VI)

The results are listed in Tables 21 and 22 As can be seen, no significant differences were obtained between subjects below the age of 40, and those who were 40 or more (Nor was any significant difference found when sub jects below the age of 30 were compared with those above the age of 50) There was no significant difference in reaction between males and females

TABLE 21 *Matched non diabetic subjects The results of the γG globulin staining in subjects $<$ 40 years and $\geqslant$ 40 years of age and in males and females*

Subjects studied	γG globulin staining (Grade) 1	2	3	4	Total No/series	Chi-square value ($x = 3 8$)	t value ($t_{0 025} = \pm 2$)
$<$ 40 years	22	7	0	0	29		
$\geqslant$ 40 years	13	8	0	0	21	1 1	0 6
Males	19	8	0	0	27		
Females	16	7	0	0	23	0 2	0
Total No/Grade	35	15	0	0	50		

The mean systolic and diastolic blood pressures were practically identical in the groups of grade 1 and grade 2 immunofluorescent staining

Patients with diabetes mellitus

The possible influence of various factors of the diabetic disease on the localization of γG globulin in small dermal vessel walls was studied and the results were treated in the same way as has been described above for the PAS-staining The results are listed in Table 23

γG globulin was found more often localized in the small vessel walls in the series of diabetic patients than in the series of matched non diabetic subjects In diabetics the specific fluorescence was frequently strong These differences were statistically significant (Test 1 and 2)

It was not possible to show any difference between non diabetic subjects who knew of diabetes among close relatives and those who did not know of such an occurrence The former group was small however (Test 3)

Significant differences were noted in the localization of γG globulin in diabetics with varying duration of the disease (Test 4) Positive — and often strong — specific fluorescence was the rule among patients with a known duration of 10 years or more Diabetics with a known duration of one year or less did not deviate significantly from non diabetic subjects $\chi^2 = 1.2$ (3.8) However, the difference was significant between the latter group and patients with a duration of less than five years (Test 5) or more In Figure 3 the results of the γG globulin staining are plotted against the known duration of the disease As can be seen the intensity of the specific fluorescence increased with the mean duration of the disease

When the patients with a known duration of less than 10 years were divided into two groups according to the onset of the disease — before or after the age of 40 — and the groups were compared, no significant difference was obtained (Test 7) As for the results of the PAS staining some influence of the age at the onset on the localization of γG globulin was noted, Figure 4 In the group of diabetics with an onset below the age of 20 strong specific fluorescence was fist noted after seven years duration whilst in the group with a known onset at the age of 40 or more strong fluorescence was noted within one years duration In this latter group the influence of the duration on the localization of γG globulin in the vessel walls was not so evident no significant difference was obtained when patients with a duration of less than five years were compared with those of five years duration or more [$\chi^2 = 3.33$ (5.99)] When patients who had been treated with insulin were compared with those who had not, no significant difference in the specific fluorescence in the vessel walls was seen (Test 8)

TABLE 22 *Matched non diabetic subjets Systolic and diastolic blood pressure in the different groups of γG globulin staining*

γG globulin staining (Grade)	Total No/Grade	Mean systolic pressure $\pm s_{\bar{x}}$	Mean diastolic pressure $\pm s_{\bar{x}}$
1	35	143 ± 5	88 ± 2
2	15	143 ± 6	86 ± 2
3 + 4	0	—	—
Total No	50		

TABLE 23 *The results of the staining of γG globulin in small dermal vessel walls in patients with diabetes The results were compared with those for the matched non diabetic subjects The influence of known familial occurrence of diabetes was studied in the non diabetic series In the diabetic series the influence of the known duration of the disease the age when the disease became evident and the influence of insulin treatment were studied*

Series of subjects studied	Group No	γG globulin (Grade) 1	2	3	4	Total No
Matched non-diabetic subjects	I	35	15	0	0	50
Known familial occurrence of diabetes	II	6	2	0	0	8
Familial occurrence not known	III	29	13	0	0	42
Diabetes mellitus Series A	IV	13	16	11	10	50
Diabetes mellitus Series B	V	26	19	21	5	71
Diabetes mellitus Series A + B	VI	39	35	32	15	121
Duration 0—5 years	VII	24	9	8	1	42
Duration 5—10 years	VIII	8	14	2	5	29
Duration 10—15 years	IX	5	5	10	4	24
Duration 15— years	X	2	7	12	5	26
Onset < 40 years duration < 10 years	XI	12	9	5	4	30
Onset > 40 years duration < 10 years	XII	17	14	8	2	41
Insulin treatment, diabetes < 5 years	XIII	10	3	2	0	15
No insulin treatment diabetes < 5 years	XIV	14	6	6	1	27

Test No	Groups compared	Chi square value	t value	Sign test value
1	I and IV	310 (78)		34 (27)
2	I and VI	322 (78)	80 (20)	
3	II and III		01 (20)	
4	VII VIII IX and X	358 (169)		
5	I and VII	119 (60)		
6	I and VII+VIII	—		
7	XI and XII	0.5 (60)		
8	XIII and XIV	09 (38)		

Patients with diseases other than diabetes mellitus

The results are listed in Table 24 Significantly more positive fluorescence was observed in the small dermal vessel walls *in patients* with SLE and SLE like syndromes than in non-diabetic, apparently healthy subjects When this latter series was compared with other disease groups in this study, no significant differences were seen As stated before, the small number of patients in many groups makes is necessary to judge the results with caution In the statistical treatment the grades 2—4 were combined

The ESR and the amount of circulating γ-globulin in the various groups of immunofluorescent staining of γG globulin in small vessel walls

The erythrocyte sedimentation rate (ESR) and the amount of circulating γ globulin (paper electrophoresis) were determined in most patients in the diabetes mellitus series A and in the matched non diabetic subjects One value for ESR was lost in each of the two groups Paper electrophoresis was not performed on 11 diabetics and on three non diabetics The values were grouped and compared according to the results of the fluorescent antibody staining In the four diabetic groups a one-way analysis of variance was performed while the non-diabetic groups were compared by means of the t test The mean values of the measurements obtained for the various groups are shown in Tables 25 and 26

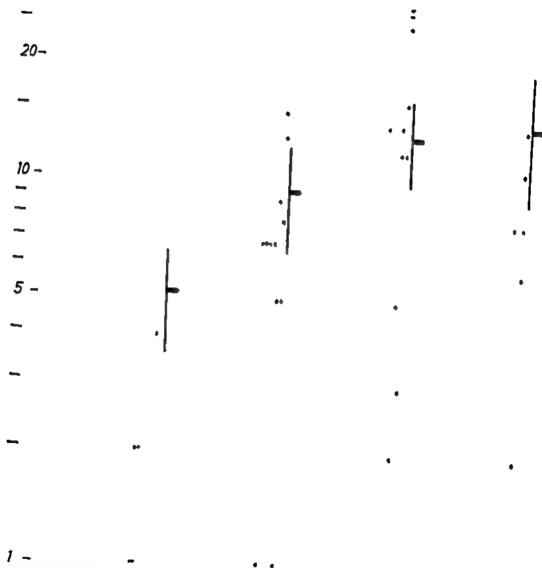
No significant differences were obtained

Summary

In a series of apparently healthy subjects weak fluorescence indicating localized γG-globulin in small dermal vessel walls was found in 30 % of the skin biopsies studied In a contrasting series of diabetic patients weak specific fluorescence was noted in 36 % and strong and bright fluorescence in 42 % of the biopsies The difference was statistically significant

FIGURE 3 *Patients with diabetes mellitus The results of the γG globulin staining of small dermal vessel walls are plotted against the known duration of the disease (logarithm scale) The mean duration and the confidence limits of the mean in each grade of γG globulin staining are shown The values are*

γG globulin (Grade)	No	Mean duration (Years)	95 % confidence limits (Years)
1	39	5 1 ± 0 8	3 6 — 6 6
2	35	9 5 ± 1 4	6 7 — 12 3
3	32	13 1 ± 1 5	10 0 — 16 2
4	15	14 3 ± 2 3	9 2 — 19 4



DURATION
years
30
20
10
5
1
γG-GLOBULIN
grade
1
2
3
4

TABLE 24 *The results of the immunofluorescence staining of γG globulin in small dermal vessel walls in patients with diseases other than diabetes mellitus The results were compared with those of the matched non diabetic series The various diagnoses in the group »Other diseases» are listed in Table 6*

Group of patients studied	γG globulin (Grade) 1	2	3	4	Total No/Group	Chi square value ($x^2 = 3\ 8$)
Glomerulonephritis	4	1	4	0	9	2 7
SLE syndrome	5	7	3	0	15	6 6
Rheumatoid disease	5	4	2	0	11	2 4
Hypertension (Mean 221/130)	6	1	2	0	[illegible]	0 1
Other diseases	24	[illegible]	2	1	33	0 1
Matched non diabetics	35	15	[illegible]	0	50	

No correlation was noted between the localization of γG-globulin in small dermal vessel walls and the age, sex, blood pressure, the ESR reaction or the amount of circulating γ globulin, respectively, of the subjects studied No difference was found between apparently healthy subjects with a known familial incidence of diabetes and subjects with out any knowledge of such an incidence

In the diabetic series the frequency and intensity of the specific fluorescence increased with the duration of the disease However, in patients with a known onset of the disease at the age of 40 or more there was no significant difference when the subjects with a duration of less than five years were compared with those with a duration of five years or more In the older

FIGURE 4 *Patients with diabetes mellitus divided into three groups according to the known age at the onset of the disease The results of the identification of γG globulin in small dermal vessel walls are plotted are shown The values are (logarithm scale) The mean duration and the confidence limits of the mean in each grade of specific fluorescence are shown The values are*

Known onset below the age of 20

γG globulin (Grade)	No	Mean duration (Years)	95 % confidence limits (Years)
1	10	7 4 ± 1 6	3 7 — 11 1
2	12	13 6 ± 2 4	8 3 — 18 9
3 + 4	20	18 4 ± 1 9	14 4 — 22 4

Known onset at the age of 40 or more

γG globulin (Grade)	No	Mean duration (Years)	95 % confidence limits (Years)
1	24	4 7 ± 0 9	2 8 — 6 6
2	15	4 7 ± 0 7	3 2 — 6 2
3 + 4	14	8 9 ± 2 1	4 4 — 13 4

TABLE 25 *The mean values of the erythrocyte sedimentation rate (ESR/hour) in the various grades of γG globulin staining of small dermal vessel walls in patients with diabetes (Series A) and matched non diabetic subjects*

Group of subjects studied	γG globulin (Grade)	Total No/Grade	ESR mm (Mean)	t or F value
Diabetes mellitus	1	13	13 ± 5	
(49 subjects)	2	15	11 ± 2	
	3	11	14 ± 3	
	4	10	20 ± 6	F = 0 9 (2 3)
Matched non diabetics	1	35	9 ± 1	
(49 subjects)	2	14	12 ± 3	
	3 + 4	0	—	t = 0 8 (± 2 0)

TABLE 26 *The mean values of the amount of circulating gammaglobulin (paper electrophoresis) in the various grades of γG globulin staining of small dermal vessel walls in patients with diabetes mellitus (Series A) and matched non diabetic subjects*

Group of subjects studied	γG globulin (Grade)	Total No/Grade	Gammaglobulin (Mean) g/100 ml	t or F value
Diabetes mellitus	1	12	0 77 ± 0 05	
(39 subjects)	2	14	0 82 ± 0 05	
	3	[illegible]	0 85 ± 0 10	
	4	7	0 81 ± 0 07	F = 0 3 (2 3)
Matched non diabetics	1	34	0 86 ± 0 03	
(47 subjects)	2	13	0 87 ± 0 05	
	3 + 4	0	—	t = 0 2 (± 2 0)

age groups strong specific fluorescence appeared earlier than in juvenile diabetics Insulin treatment did not appear to have any influence on the localization of γG globulin in small vessel walls

In patients with SLE or SLE like syndromes a significant increase in the specific fluorescence was noted when this group was compared with a group of apparently healthy subjects

CHAPTER VIII

THE RESULTS OF THE IMMUNOFLUORESCENT LOCALIZATION OF SOME PLASMA PROTEINS OTHER THAN γG GLOBULIN IN SMALL DERMAL VESSEL WALLS IN DIABETIC AND NON DIABETIC SUBJECTS

Morphology

The plasma proteins studied are discussed in Chapters III and V In most cases the morphological pattern of the specific fluorescence in small dermal vessel walls was very similar for all plasma proteins studied The description of the fluorescence given in Chapter VII can be applied to all

A few exceptions may be noted

Albumin seemed to be localized more peripherally around the vessel walls as a fluorescent cuff In such cases hardly any fluorescence was noted in the basement membrane region

More often than for γG globulin endothelial cells, or the luminar side of the cells fluoresced when albumin, transferrin haptoglobin, β_1c-globulin and fibrinogen were studied

Sometimes α_1 lipoprotein was seen stained as bright blobs or small crescents within the walls while the rest of the vessels were non fluorescent

The fluorescence was graded from 1 to 4 as for γG globulin

Differences between apparently healthy subjects and patients with diabetes melitus

The distributions of the various plasma proteins were studied in apparently healthy, matched non diabetic subjects and compared with those obtained for diabetics, Table 27

All plasma proteins studied, except albumin, were present in a higher percentage in the diabetic series the difference was significant for transferrin, β_1c-globulin and γM globulin (Grades 2—4 were combined when albumin, transferrin fibrinogen, γA- and γM globulin were tested and Grades 3—4 when α_1 lipoprotein and β_1c globulin were tested Because of the smallness of the groups, α_1 acid glycoprotein, haptoglobin and β lipo protein were not tested statistically) It must be emphasized that the number of subjects studied in some groups was too small to permit any test and to allow any conclusions to be drawn

However, some observations may be pointed out

1 With the method used most plasma proteins studied were observed in small dermal vessels in singular, apparently healthy subjects

2 The same plasma proteins — with the exception of albumin — were seen more frequently in small dermal vessel walls in patients with diabetes

3 The specific fluorescence indicating the presence of these proteins was more

TABLE 27 *The results of the immunofluorescent localization of various plasma proteins in small dermal vessel walls in apparently healthy non diabetic subjects and in patients with diabetes mellitus The Chi square values refer to the statistical comparison of the results in the two series of investigated subjects*

Plasma protein studied	Non diabetic subjects Fluorescence (Grade) 1	2	3	4	Total No	Diabetic patients Fluorescence (Grade) 1	2	3	4	Total No	Chi square value
Albumin	35	14	0	0	49	62	16	0	0	78	1 1 (3 8)
α_1 acid glycoprotein	21	0	0	0	21	16	2	1	0	19	—
α_1 lipoprotein	20	7	4	0	31	15	9	7	6	37	5 2 (6 0)
Haptoglobin	19	1	0	0	20	14	2	1	0	17	—
Transferrin	12	1	0	0	13	10	7	1	0	18	5 0 (3 8)
β lipoprotein	18	1	0	0	19	18	3	1	0	22	—
β_{1c} globulin	37	7	0	0	44	44	20	8	1	73	9 2 (6 0)
Fibrinogen	43	3	0	0	46	59	12	1	0	72	3 2 (3 8)
γG globulin	35	15	0	0	50	39	35	32	15	121	32 2 (7 8)
γA globulin	34	5	0	0	39	41	13	3	1	58	3 6 (3 8)
γM globulin	43	1	0	0	44	45	14	5	1	65	13 7 (3 8)

TABLE 28 *Patients with diabetes The results of the immunofluorescence staining of some plasma proteins in small dermal vessel walls in patients with a known duration < 10 years and > 10 years*

Plasma protein studied	Duration < 10 years (Grade) 1	2 — 4	Total No	Duration > 10 years (Grade) 1	2 — 4	Total No	Chi square value (x^2 = 3 8)
Albumin	38	7	45	24	9	33	1 6
α_1 acid glycoprotein	11	0	51	5	3	8	—
α_1 lipoprotein	11	7	18	4	15	19	6 2
Haptoglobin	10	2	12	4	1	5	—
Transferrin	7	4	11	3	4	7	—
β lipoprotein	9	3	12	9	1	10	—
β_{1c} globulin	27	15	42	17	14	31	0 7
Fibrinogen	36	6	42	23	7	30	1 0
γA globulin	31	6	37	10	11	21	8 5
γM globulin	33	6	39	12	14	26	10 8

frequently strong in patients with diabetes

In all these respects, the tendency of the observations was similar to that already observed when the distribution of γG globulin was studied

The influence of the duration of diabetes

The diabetic patients were divided into two groups, those with a known duration of less than 10 years and those with a duration of 10 years or more When the two groups were compared, Table 28, significantly more positive results were obtained in the group with a longer duration when α_1 lipoprotein, γA and γM globulins were studied In all proteins studied except β lipoprotein the same tendency was observed

Summary

In apparently healthy non diabetic subjects specific fluorescence indicating localized haptoglobin transferrin, β-lipoprotein, β_{1C} globulin, fibrinogen, γA and γM globulins in small dermal vessel walls was noted only in one or in very few of the skin biopsies investigated Albumin and α_1 lipoprotein was identified in about 30 % of the investigated biopsies, or to about the same extent as γG globulin In some specimens very strong fluorescence indicating localized α_1 lipoprotein was noted

The frequency of specific fluorescence indicating localized albumin was similar in diabetic and non diabetic subjects Other plasma proteins studied were seen more frequently in diabetic than in non diabetic subjects In the former group the specific fluorescence was sometimes strong

There was an increase in the intensity and frequency of the specific fluorescence of all plasma proteins studied with increasing duration of diabetes, with the exception or β lipoprotein

The pattern of the observed specific fluorescence was thus very similar to that indicating localized γG globulin in the vessel walls

CHAPTER IX

GENERAL DISCUSSION

Observation in PAS treated sections

The observed thickening of the PAS positive dermal vessel walls in diabetic patients is in agreement with the results of previous investigations by others using the same technique (cf Table 1) A slight thickening was often noted in non-diabetic, apparently healthy subjects and in patients with diseases other than diabetes A heavy (grade 4) thickening of the vessel wall was with one exception, only seen in diabetic patients, namely in a 69 year old woman with high blood pressure, 280/100 and a normal oral glucose tolerance test The observed connection of the diabetic state with a heavy PAS positive thickening of small vessel walls is also in agreement with earlier observations (cf Save-Soderberg et al, 1967) As stressed previously, it is not possible in light microscopic studies of this kind to determine whether the thickening is caused by an increased width of the basement membrane proper or of the collagen containing perivascular cuff or whether both these structures are involved (cf Friederici et al 1966 Pardo et al 1966 Siperstein et al 1966)

In the PAS treated sections it was observed that the wall thickness increased with the mean duration of diabetes This too is in agreement with the results obtained by others who studied large series of skin biopsies (cf McMillan et al, 1966 Otto et al, 1966, Save-Soderbergh et al, 1967) The positive correlation between the increasing width of the vessel walls and the duration of the disease may not, however, become evident if only patients with adult onset diabetes are studied, *vide infra*

The results of this study seem to indicate that heavy thickening of the vessel walls could be noted early (within one year) in diabetic patients with a known onset of the disease after the age of 40 In juvenile diabetics with an onset below the age of 20 a similar thickening was not seen until after eight years The anamnestic data of patients in several previously published studies on small vessel changes in biopsy specimens are incomplete, and the age at the onset of the disease is not mentioned In most series however, the majority of patients were more than 40 years old In those series, where the onset of the disease is stated, only a few patients with an onset below the age of 30 were studied This may contribute to the difficulty in elucidating the possible influence of the duration on the increase of the vessel wall thickness In genetically prediabetic subjects (both parents were overt

ly diabetic, the fasting blood sugars and oral glucose tolerance test were claimed to be within the normal range) Siperstein *et al* (1966) found ultra structurally a thicker basement membrane in muscle capillaries than in the normal subjects studied The difference was statistically significant In overtly diabetic subjects the membranes were significantly thicker than in the pre diabetic individuals studied This might indicate an influence of the duration of the disease on the basement membrane width Yet, in the overtly diabetic subjects the thickness was found to be unrelated to the duration of the disease Neither the age of the subjects studied, nor the age at the onset of the disease are however, included in this study

Lazarow (1967) found a positive correlation between the duration of diabetes and the width of the glomerular capillary basement membrane, studied under the electron microscope He found too, that in adult diabetics of recent onset the basement membrane was thicker than in juvenile diabetics of the same duration Furthermore in young diabetics the basement membrane width did not differ from that of non diabetic subjects Østerby Hansen (1966) did not find any significant difference in the glomerular peripheral basement membrane width of recent onset juvenile diabetics and of non diabetic subjects The thickness increased however with the increasing duration of the disease (Østerby Hansen 1967) The present author s observations are consistent with these studies

Only two published studies on skin biopsies are devoted to small blood vessel changes in juvenile diabetics (Otto *et al* 1967, Save Soderbergh *et al* 1967) In both series a positive correlation was found between the thickness of the small vessel walls and the duration of the disease The present results conform to this

The influence of the age at the onset of diabetes on the observed morphological changes in the small blood vessels may be more apparent than real, however The difference in this respect between juvenile and adult onset diabetics may be due to the high frequency of unrecognized diabetes of long duration in older age groups (*cf* Keen 1966, Anderson, 1966)

In patients with rheumatoid arthritis and systemic lupus erythematosus (SLE) the small vessel walls were also found to be thicker than in apparently healthy subjects Like diabetes mellitus these diseases are chronic ones and furthermore they are both inflammatory The basic morphological similarities, observed by Bloodworth (1965) in kidney sections under the electron microscope between diabetic glomerulosclerosis glomerulonephritis and SLE may be noted in this connection However, the groups of patients investigated in the present study are too small to allow any further conclusions to be drawn

Immunohistochemical investigations

Despite the various sources of specimens used, the various preparatory procedures and staining techniques the results of immunofluorescent investiga

tions of the possible localization of γ-globulin in diabetic vessel walls indicate that this protein can be identified primarily in the region of the basement membranes in these vessels (*cf* Tables 3 and 4) Other plasma proteins are studied to a lesser extent (albumin, fibrinogen, low density lipoproteins, fractions of complement) but the results are inconclusive

In the majority of investigated cases, however, reports on clinical data are lacking or insufficient The series containing the largest number of cases were made in retrospect on sections obtained from tissue blocks in the files of Departments of Pathology (Berns *et al* 1962, 1964, Blumenthal *et al*, 1966) It is therefore, difficult to correlate the findings with clinical data and the natural course of diabetes

When the studies are made in tissues from kidney, eye, pancreas or amputated legs it is obvious that comparative investigations of identical tissues from healthy individuals are practically impossible The importance of adequate control series of this kind however is underlined by the results of the present investigation in apparently healthy subjects γG globulin was identified in small vessel walls in almost ⅓ of the subjects studied albumin and α_1 lipoprotein were shown in approximately the same degree and haptoglobin transferrin β lipoprotein β_{1C} globulin, fibrinogen γA and γM globulins in only one or very few of these healthy subjects (Table 27) Fibrinogen, β_{1C}-globulin and γ-globulins may all take part in immunological processes The results of this study stress the importance of studying more than one or a few of these proteins, when the primary aim of the study is to investigate whether histological changes are of immunogenic origin, or not Conclusions drawn from such studies may be valid but are not necessarily so The normal passage of serum proteins from circulating blood into the surrounding connective tissue (*cf* Mancini, 1965), the increased leakage of proteins through the vessel walls and the possible accumulation of these proteins in damaged walls in many types of vascular lesions must be considered (*cf* Cotran & Majno, 1964, Zweifach, 1964)

In the present investigation a marked relationship was noted between the wall thickness observed in PAS treated sections, and the observed localisation of γG globulin in small dermal vessel walls The techniques performed do not allow any absolute quantitations of the results to be made It cannot be stated whether the observed accumulation of γG globulin and some other serum globulins are primary or secondary to the observed thickening of the vessel walls

Localized γG globulin was noted significantly more often in the total series of diabetic patients than in healthy individuals The specific fluorescence was more frequently intense in the diabetic series There was a positive correlation between the duration and the frequency and intensity of the specific fluorescence A similar difference was noted between the series of juvenile and adult onset diabetics when γG globulin was studied as was seen in PAS treated sections

(*vide supra*) Here too, the explanation may be that in older age groups the disease often remains unrecognised for long periods

It has been supposed that aging is associated with a rising incidence of autoimmune reactions and this maybe more so in diabetic subjects (*cf* Blumenthal & Berns, 1964 Ungar *et al*, 1967) »Bound» autoantibodies might then accumulate in the vessel walls and contribute to the strong specific fluorescense indicating γG globulin, which was observed in short term adult onset diabetics However, γG globulin appeared with equal frequency in the vessel walls of healthy subjects above the age of 50 and below the age of 30 With the exception of albumin, the vascular localization of the other proteins studied seemed to be dependent on the duration of diabetes in the same way as that of γG globulin As stated earlier, it therefore seems unjustified at present to draw too definite conclusions merely based on the localization of γG globulin in the vessel walls

Localized γG globulin was significantly more often to be found in the vessel walls of patients with SLE than in healthy subjects The results are in keeping with those obtained by others studying the localization in *e g* the lesioned skin and in the kidneys (*cf* Chapter II and Tables 2 and 3)

The immunogenic theory of pathogenesis

In many studies based of e cal staining methods (*cf* 1949, Ashton, 1957, Lendrum 1963) the accumulation of plasma protein in the vessel walls has been assumed in diabetes mellitus These methods cannot, however, with certainty identify single proteins The fluorescent antibody technique offers the possibility of identifying small quantities of specific proteins in tissue sections (*cf* Mellors 1959, Nairn, 1962)

The theory was put forward earlier (Starck 1954, Gellman *et al*, 1959) that vascular lesions in diabetes might be caused by immunological reactions and it was substantiated by the identification of γ globulins associated with vascular lesions in diabetic subjects (Odin & Tornblom, 1959, *cf* Table 2) The validity of the theory seemed to be further strengthened by the observations of Berns *et al* (1962) and others (Table 4) that conjugated insulin was bound *in vitro* to the same sites in vascular walls, where *in vivo* localized γG globulin could be found Furthermore conjugated anti insulin serum was bound *in vitro* in similar sites as conjugated insulin Insulin was thought to be the antigen in those antigen antibody reactions that was thought to be of pathogenic importance in the development of the small vessel lesions in diabetes The insulin could either be a »changed» endogenously produced insulin or introduced into the body during the insulin treatment (*cf* Blumenthal et al, 1964)

The present author s own investigations of skin biopsies treated with conjugated pr ulin, seem to indicate » f insulin to dermal structures of

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The present author s own investigations of skin biopsies treated with conjungated pig insulin seem to indicate that the »binding» of insulin to dermal vessel walls and other structures of

the dermis is dependent on the fixation methods used during the preparation of the sections studied (These investigations are soon to be published) In formalin fixed sections a more selective binding of the insulin to vascular basement membranes — seemingly more so in diabetic subjects — and to dermal connective tissue fibres was noted If the sections were fixed in cold ethanol according to the modified Sainte Marie method the conjugated insulin became bound almost selectively to all kinds of cells in the sections while almost no fluorescence was seen in the basement membrane regions No differences were observed between diabetic and non diabetic subjects The present author therefore considers that at the moment the results of immunohistochemical investigations of conjugated insulin and anti insulin serum cannot be referred to as support for the theory that insulin is the antigen in the supposed antigen antibody complexes localized in small vessel walls in diabetic subjects

SUMMARY

On 122 patients with diabetes mellitus skin biopsies were performed and the small blood vessels were studied in PAS treated sections With the aid of the fluorescent antibody technique the localization of γG globulin in the vessel walls was investigated 50 of the diabetic patients were matched with 50 non-diabetic subjects on whom similar studies were performed

In the diabetic series a thickening of the PAS positive vessel walls was seen significantly differing from the wall thickness in non diabetic subjects There was a positive correlation between the increase of the wall thickness and the duration of diabetes This was best seen in patients with a known onset of diabetes below the age of 20

Similarly γG globulin was identified significantly more frequently localized in the vessel wals of diabetic than of non diabetic subjects There was a positive correlation between the frequency and intensity of the specific fluorescence and the duration of the disease This was best seen in patients with a known onset of the disease below the age of 20

The localization of some other plasma proteins in the vessel walls was to a lesser extent also studied using the fluorescent antibody technique Albumin was found with the same frequency in diabetic and non diabetic subjects Other plasma proteins studied (α_1 acid glycoprotein α_1 lipoprotein haptoglobin transferrin β lipoprotein β_{1C} globulin fibrinogen γA and γM globulins) were found more often in diabetic than in non diabetic subjects and more often in long term than in short term diabetes

The PAS reaction and the localization of γG globulin in the dermal vessel walls were also studied in 77 patients with various diseases other than diabetes When compared with healthy subjects significantly thickened vessel walls were only observed in patients with rheumatoid arthritis and systemic lupus erythematosus (SLE) and a significantly higher frequency of localized γG globulin was only seen in patients with SLE

Professor Astrid Fagraeus Statens Bakteriologiska Laboratorium Stockholm first introduced me to the fluorescent antibody technique and the various problems connected with this method She also generously supplied me with some of the antisera used in this investigation I have also gratefully received some valuable antisera from Professor Sven Bergman Institute of Clinical Bacteriology Umea Dr Bengt Lundh Department of Medicine Lund and Dr Hans J Muller Eberhard Scripps Clinic & Research Foundation La Jolla Calif

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A



B



C



D

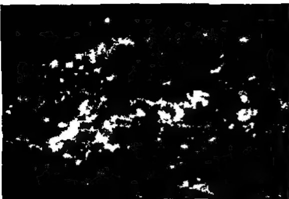
PLATE III. *Skin biopsies. Indirect immunofluorescent staining of γG globulin by means of rabbit anti-human γG globulin serum + conjugated goat anti rabbit globulin serum. The intensity of the fluorescence is graded from 1 to 4. Orig. magn. x 400*

A. No 141. Age 22. Healthy. Grade 1

B. No 19b. Age 21. Diabetes mellitus, duration 13 years. Grade 2

C. No 25. Age 28. Diabetes mellitus, duration 10 years. Grade 3

D. No 4. Age 35. Diabetes mellitus, duration 22 years. Grade 4



A



B



C



D

ATE IV Skin biopsies Localization of various plasma proteins by aid of indirect immunofluorescent staining All stainings were performed with the same conjugated goat anti rabbit globulin serum The unconjugated antisera used were A Serum No 2 B Serum No 3 C Serum No 1 D Serum No 7 listed in Table 8 Orig magn x 400

A No 176 Age 70 Diabetes mellitus duration 5 years Specific fluorescence indicating albumin diffusely localized in connective tissue

B No 194 Age 19 Diabetes mellitus duration 9 years Specific fluorescence indicating α_1 acid glycoprotein localized in perivascular cells

C No 128 Age 33 Diabetes mellitus duration 20 years Specific fluorescence indicating α_1 lipoprotein localized in small dermal vessel walls and in the superficial layer of the dermis

D No 238 Age 46 Healthy Specific fluorescence indicating β lipoprotein coating the endothelial cells of a small artery and vein



A



B



C



D

PLATE V *Skin biopsies Localization of various plasma proteins by aid of indirect immunofluorescent staining All stainings were performed with the same conjugated goat anti rabbit globulin serum The unconjugated antisera used were A. Serum No 9 B Serum No 10 C Serum No 14 D Serum No 15 listed in Table 8*

A No 5 *Age 36 Diabetes mellitus duration 29 years Specific fluorescence indicating* $\beta_{1}c$ *globulin localized in small dermal vessel wall and immediately beneath the epidermis Orig magn × 400*

B No 176 *Age 70 Diabetes mellitus duration 5 years Specific fluorescence indicating fibrinogen coating endothelial cells in small vessels*

C No 179 *Age 70 Healthy Specific fluorescence indicating γA globulin in the area of the dermal epidermal junction Orig magn × 160*

D No 142 *Age 27 Diabetes mellitus duration 8 years Specific fluorescence indicating γM globulin mainly localized in small vessel wallas Orig magn × 160*



A



B



C



D

PLATE VI Skin biopsies Indirect immunfluorescent demonstration of γG globulin Serum No 12 (Table 8) + conjugated goat anti rabbit globulin serum

A. No 4 Age 35 Diabetes mellitus duration 22 years Orig magn x 160

B No 130 Age 28 Diabetes mellitus duration 12 years Orig magn x 160

C No 196 Age 24 Diabetes mellitus duration 13 years Specific fluorescence in small dermal vein no fluorscence in small artery Orig magn x 160

D No 25 Age 28 Diabetes mellitus duration 10 years Strong specific fluorescence in small vessels close to sweat glands Orig magn x 400